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A Study of the Viscometric Behaviour of Branched and Linear Dextran Polyelectrolytes

by

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-----to m whose unfailing patience,

dedication and encouragement

during the compilation of this

work made it easier to

overcome the encountered

trials and tribulations-----

ABSTRACT

Various linear and branched dextrans of known molecular weight and structure were converted into polyelectrolytes -- dextran sulfates and carboxymethyl dextrans -- of differing degrees of substitution by methods designed to minimize degradation, whether by chain scission or branch hydrolysis. Reduced viscosities of these polyelectrolytes were determined in N/1000 and N/2000 KCl at various concentrations. Effects, on the reduced viscosity-concentration curves, of molecular weight and molecular weight distribution, of degree of substitution, of degree of branching, and of the ionic strength of the solvent were determined, using various criteria developed for the purpose. It is evident that with proper control of the other factors, differences in degree of branching in dextran polyelectrolytes (and their parent polymers) can be detected viscometrically. For example, the reduced viscosity-concentration curves of linear and branched dextran polyelectrolytes, prepared from dextrans of the same intrinsic viscosity and substituted to the same degree, exhibited a characteristic "cross-over" which can only be due to differences in branching and which are attributed to the higher local charge density within the branched macromolecular coil.

The need to determine whether the branches are hydrolytically removed during the sulfation process prompted proton magnetic resonance spectroscopy studies of linear and branched dextrans and dextran sulfates. These studies led to the development of a method for the detection of branches in dextran samples which should be applicable to other polysaccharides. The method showed that, as desired, no hydrolysis of branches occurred during sulfation.

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A. GENERAL INTRODUCTION

Statement of the Problem

The physical properties of a polymer, whether in bulk or in solution, may depend to a marked degree on the nature and extent of branching in the polymer molecules. Over the past twenty years, therefore, considerable effort has been expended in attempts to develop convenient, reliable methods of detecting, characterizing, and determining the extent of branching in polymers. Of the methods based on physical measurements -- and these are preferred because of their greater convenience -- several viscometric methods have enjoyed a considerable degree of popularity. None of these, however, is wholly satisfactory.

In a pioneering statistical study of polymers in solution, Zimm and Stockmayer (1) concluded that, in solution, branched polymer molecules are less extended (i.e. occupy a smaller volume) than the corresponding linear molecules of the same molecular weight. Using measurements of viscosity and light scattering, Thurmond and Zimm (2) verified these conclusions experimentally and demonstrated how measurements of intrinsic viscosity $[\eta]$ and weight-average molecular weight $\bar{M}_w$ might be used in the detection and estimation of branching.

For a particular polymer-solvent system, a sample of branched polymer has a lower intrinsic viscosity than a sample of linear

polymer of the same weight-average molecular weight. This is illustrated in Figure 1 in which are plotted $[\eta] - \bar{M}_w$ data (2) for branched polystyrenes (copolymers of styrene and divinylbenzene) and linear polystyrenes. Thurmond and Zimm (2) worked with a synthetically branched polymer; similar behaviour has been observed with the naturally occurring polymer dextran (see Figure 2) and, indeed, with a wide variety of polymers in which branching was known to occur.

In Figures 1 and 2 -- and these are typical of many other systems -- a difference in the intrinsic viscosity of branched and linear polymers, which is a measure of the extent of branching, is not evident in the low molecular weight region. On this basis, one might conclude that branching does not exist in the lower molecular weight polymers.

It may well be, however, that there is branching in these polymers but that the viscometric method (2) is not sufficiently sensitive to detect it.

A number of studies support the idea that branching <u>does</u> extend into the low molecular weight regions (3-5). For example, Senti <u>et al</u> (3) found that, in periodate oxidation of B-512 dextran samples, the amount of formic acid produced per anhydroglucose unit (see Section B III) was independent of the molecular weight from 9.5×10^6 down to 1.8×10^4 -- even though the intrinsic viscosity difference between linear and branched dextran apparently vanishes below a molecular weight of 100,000 (see Figure 2).

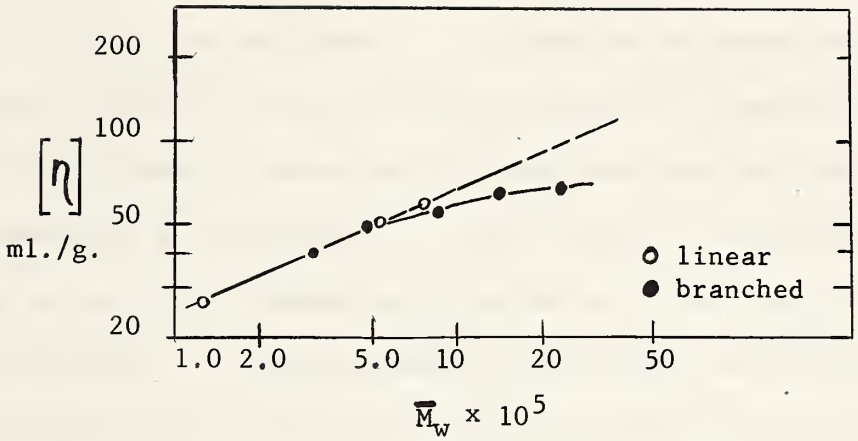


Figure 1. Logarithmic plot of $[\eta]$ vs $\bar{M}_w$: linear polystyrene and branched polystyrene (divinylbenzene-co-styrene) in butanone-propanol.

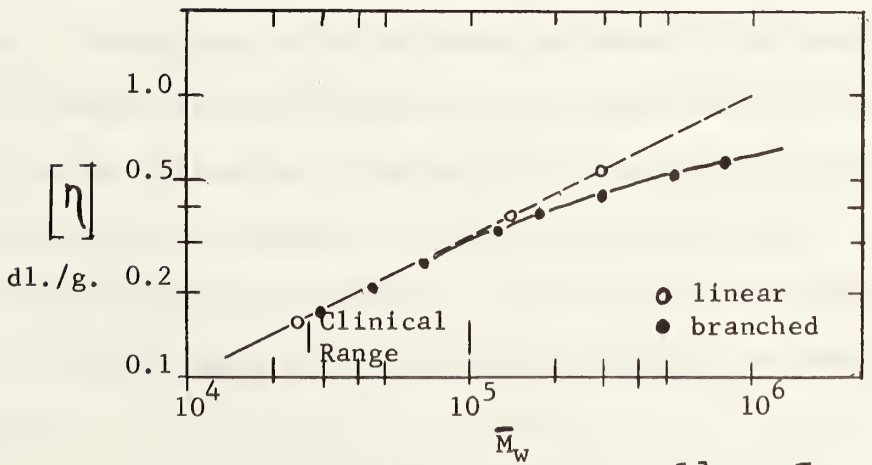


Figure 2. Logarithmic plot of $[\eta]$ vs $\bar{M}_w$: linear and branched dextran in water.

Annoyingly insensitive though it is, this method of detecting branching is the most generally applicable and most reliable of the physical methods yet available. Efforts to increase its sensitivity, especially in the low molecular weight region by exaggerating differences in the viscosity behaviour of linear and branched species, might therefore prove valuable. This thesis is concerned with an attempt to do this by converting branched and linear polymers into polyelectrolytes. Dextran samples, both linear and branched, were converted into the polyelectrolytes dextran sulfate and carboxymethyl dextran, and the viscosity behaviour of the linear and branched polyelectrolytes was studied and compared.

In bringing about a desired modification in a high polymer, there is always a possibility of causing undesired modifications. In particular, the conversion of dextran to polyelectrolyte might well cause the removal of branches by hydrolysis at the branching sites. Comparison of the infrared spectrum of the branched carboxymethyl dextran with the spectrum of the parent dextran is feasible and gives an indication of whether extensive branch hydrolysis had occurred during conversion. Such a direct comparison is not possible with the dextran sulfates. For this reason a proton magnetic resonance study was carried out on dextrans and their dextran sulfates.

B. HISTORICAL INTRODUCTION

I. Viscosity

To facilitate discussion in and understanding of the succeeding chapters, some terms and concepts widely employed in the literature dealing with the dilute solution viscometry of polymers will be briefly dealt with in this section. For more extensive reviews, the following may be consulted: Tompa (6), Tanford (7), Allen (8), Flory (9), Alexander and Block (10), Frisch and Simha (11).

(a) Nomenclature

When a solution is made up of a high polymer in a solvent, it is found that the viscosity of the solution is much greater than that of the pure solvent, even when the polymer concentration is low.

If η is the viscosity of the solution and η_0 the viscosity of the solvent, the increase due to polymer is $\eta - \eta_0$. The relative increase in viscosity (the increase relative to the viscosity of the solvent) is therefore $\frac{\eta - \eta_0}{\eta_0}$, or $\eta_r - 1$ if the viscosity ratio η/η_0 is designated η_r. (The viscosity ratio is often called the relative viscosity, even though it is a dimensionless quantity.) The quantity $\eta_r - 1$ is frequently called the "specific viscosity" and designated η_{sp}. Thus

$$\frac{\eta - \eta_0}{\eta_0} = \frac{\eta}{\eta_0} - 1 = \eta_n - 1 = \eta_{sp} \qquad \ldots 1$$

The relative increase of the viscosity is, as might be expected, concentration dependent; in an attempt to make the viscosity increase due to dissolved polymer more nearly a characteristic parameter of the polymer molecule, the specific viscosity is divided by the concentration of the polymer in solution. This new function η_{sp}/c, called the reduced viscosity, is essentially independent of concentration if the molecular weight of the polymer is low, but with increasing molecular weight there is increasing concentration dependence. This is attributed to interaction between polymer molecules. The reduced viscosity freed of such an effect, that is, the value characteristic of the polymer molecules when they are so far apart in solution that their interaction is negligible, is obtained by extrapolation of the η_{sp}/c vs. c line to infinite dilution (zero concentration). This value is usually called the intrinsic viscosity and is given the symbol $[\eta]$. Thus

$$[\eta] = \lim_{c \to 0} \left(\frac{\eta_{sp}}{c} \right) \qquad \ldots 2$$

If, as is customary, the concentration is expressed

as grams of polymer per deciliter of solution (g./dl.), both the reduced viscosity and the intrinsic viscosity are in the units deciliters per gram and have the dimensions of a specific volume. Intrinsic viscosity or reduced viscosity, then, can be interpreted as a volume pervaded by one gram of the polymer molecules in solution as it flows under the shearing force. The reduced viscosity is the average volume (per gram) pervaded in flow by polymer molecules at finite concentration, and the intrinsic viscosity is the average hydrodynamic specific volume when the molecules are far enough apart to make their contributions individually. The (time-average) shape of the molecule in flow, as well as its size, will therefore affect the reduced viscosity or the intrinsic viscosity.

(b) Flory-Fox Viscosity Equation

Many attempts have been made to calculate the intrinsic viscosity of polymer solutions (12-16). The Flory-Fox (9) (16) treatment which introduced the molecular expansion factor α (see Glossary, G1), has been the most successful to date. For linear flexible molecules, these workers related the intrinsic viscosity to molecular parameters by the relationship

$$[\eta] = K M^{1/2} \alpha^3 \qquad \ldots 3$$

where K, a constant, is equal to $\Phi' \left(\frac{\overline{r_0^2}}{M}\right)^{3/2}$

Φ' is a numerical constant of approximate value 3×10^{21} known as the Flory Constant

$\overline{r_0^2}$ is the unperturbed mean square end-to-end distance (see G2) of the polymer chain

α is the ratio of the perturbed or expanded average radius of gyration to the unperturbed average radius of gyration (see G3)

M is the molecular weight of the polymer.

The role that equation 3 has played in the theory of viscometric branch detection will be discussed in a later section (B II c).

(c) Viscosity and Shear Rate

Newton defined the viscosity coefficient by the relationship

$$\tau = \eta D \qquad \ldots 4$$

where τ is the shearing stress, D is the velocity gradient or rate of shear, and η is the "viscosity coefficient" or, more briefly, the viscosity. A liquid for which η is independent of shearing stress (and velocity gradient) is said to be a Newtonian liquid and to exhibit Newtonian behaviour. Any liquid whose viscosity coefficient is dependent upon the shearing stress or the velocity gradient is therefore a "non-Newtonian liquid."

Generally, long rigid polymer molecules in solution cause the system to exhibit non-Newtonian behaviour.

Under high shearing stresses such molecules tend to become oriented in the direction of flow of the solution. Hence the viscosity and the relative viscosity functions (η_r, η_{sp} $\eta_{sp/c}$) decrease with increasing shear stress.

Non-Newtonian behaviour is also observed with solutions of high molecular weight polymers whose molecules are flexible. For example, Sharman, Sones and Cragg (17) and Hartmann and Patat (18) have observed shear dependence of $\eta_{sp/c}$ in solutions of polystyrene of molecular weight 1×10^7. Extremely slight shear dependence of 9.8×10^6 molecular weight dextran at moderately high concentrations has been observed by Cragg and Van Oene (19). Shear dependence in solutions of flexible molecules is attributed to combined deformation and orientation of the flexible molecular "coils" and sometimes to prior disentanglement of the coils: solutions of completely flexible low molecular weight polymer molecules would, however, be Newtonian.

It is obvious that in viscosity studies of polymer systems, the shear stress is a factor that must be considered. If it can be shown that the system is Newtonian, this factor may be ignored. If the system is non-Newtonian, values of the viscosity function must be obtained at some standard shear stress (preferably zero stress) in some suitable way.

II. Viscosity and Branching

Since this study is concerned with the viscosity behaviour of branched macromolecules, it is of interest to review viscometric methods which are currently used for the detection of branching in polymeric compounds.

(a) Huggins' k′ and Branching

Early work on polymer solution viscosity indicated that the reduced viscosity of a polymer system was not independent of the concentration of polymer. This dependence prompted many attempts to produce empirical and theoretical equations to describe the concentration dependence of the reduced viscosity. In 1942, Huggins (20) proposed the following equation

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]c \qquad \ldots 5$$

for dilute solutions of polymers. Huggins took as his starting point the equation that Stokes derived for a solution of solid spheres. He introduced the "constant" k' into his calculations to provide for differences in the model chosen for polymer molecules and Stokes' spheres. Because this equation, of the many proposed, best fitted the data at low concentrations, it was widely accepted.

Simha (21) in 1950 predicted theoretically that k' should be dependent on a number of factors, one of which is of particular interest to this study -- namely, branching.

In the same year, Walker and Winkler (22) produced the first definite experimental evidence that k' can be affected by branching. These workers compared the viscosity behaviour of linear polystyrene with that of a non-linear copolymer made from styrene and divinylbenzene (DVB). (Introduction of divinylbenzene causes considerable branching and crosslinking.) The linear polymer, polystyrene, possessed a k' value of 0.38, but the co-polymers had k' values varying from 0.50 to 0.80, the value being larger the greater the proportion of divinyl-benzene in the polymerizing mixture. Since then, increase of k' with branching has been observed with many systems, but usually the nature of the branching has been ill-defined.

Manson and Cragg (23), in an attempt to clarify understanding of the relation between k' and branching, studied polymers with relatively well-defined comb-type branching (polystyrene branches attached to a polystyrene backbone) produced with the aid of newly-developed grafting techniques. Polymer samples containing from two to eighteen branches per polymer molecule were prepared, and k' values determined. No significant variation in k' with branching could be detected. They concluded that k' is not affected by comb-type branching. They also pointed out that in those

polymers for which an increase of k' with branching had been observed, the branching would be of the "bushy" (or branch-on-branch) types. The most notable example is afforded by the starches; k' for amylopectin (which is extensively branched in the "bushy" fashion) is 1.49, whereas k' for amylose, the linear form, is 0.58. The effect of branching on k' is therefore dependent on the nature of the branching. Since 1956, little progress has been made in developing k' as a measure of branching.

Lack of interest in k' as a measure of branching is probably due, in part, to the complex dependence of k' on many other factors. Simha (21) predicted that k' should also be dependent upon the nature of the solvent. This influence of solvent upon k' has been many times observed; it is of comparable magnitude to the influence of branching and is in the same sense ~~direction~~. Tompa (6) has quoted experimental results that indicate k' is slightly dependent on molecular weight (as Simha (21) also predicted) and hence on molecular weight distribution. Heterogeneity in the polymer sample tends to increase k'. Molecular association of polymer molecules in solution does likewise (24).

With no ready means of separating the different effects which operate to increase it, k' remains an empirical parameter which can be used only in a qualitative manner as an indication of branching.

(b) Mark-Houwink Exponent "a" and Branching

In the early development of polymer chemistry, Staudinger (25) suggested

$$\frac{\eta_{sp}}{c} = KM \qquad \ldots 6$$

as an empirical relationship between viscosity and molecular weight of high polymers. Further investigations by, amongst others, Kuhn (26), Mark (27) and Houwink (28) showed that the relationship should be

$$[\eta] = KM^a \qquad \ldots 7$$

This equation is valid generally for linear polymers: a plot of log $[\eta]$ vs log M gives a straight line whose slope is "a" and intercept on the ordinate axis is log K. In branched polymers $[\eta]$ is less than for the linear polymer of the same molecular weight and the log $[\eta]$ – log M curve falls below that for the linear polymer, usually swinging away from it at higher and higher molecular weights (see Figure 1). This suggests that the exponent "a" might be used as a measure of the extent of branching.

The copolymer styrene-divinyl benzene of Thurmond and Zimm (2), which would have trifunctional branch points and possibly crosslinks, showed decreasing "a" values as molecular weight increased (see Figure 1). Johnson and

Wolfangel (29) also obtained lower "a" values for such copolymers. Granath (30) observed that the "a" value for dextran, which was suspected to be highly branched because of the low percentage (69.7) of 1,6 linkages (see Section B III) was considerably less than the "a" value of a dextran with a higher percentage (94.2) of 1,6 linkages. Indeed, such decrease of "a" with branching is invariably observed. However, the value of "a" can vary from 0.5 to 0.8 for flexible linear macromolecules, depending on the solvent. This means that in order to determine whether "a" is less for a particular polymer system than for the corresponding linear polymer system, the value for the linear polymer system must be measured in the same solvent and at the same temperature. The need for measurements with a linear polymer, however, can be circumvented if the intrinsic viscosity-molecular weight relationship for the branched polymer is determined in a theta solvent (see G4). Under θ conditions, "a" has, according to both theory and experiment, a value of 0.50 for all flexible linear macromolecules. Therefore, since "a" is always less for branched polymers, a value of "a" less than 0.50 in a theta solvent may be interpreted as an indication of branching. For example, exponents of 0.21 and 0.28

have been experimentally observed in θ solvents for polymethyl siloxanes (31) which were purposely branched trifunctionally.

As might be expected, "a" is dependent upon factors other than the extent of branching: both temperature and solvent have pronounced influences on "a". Data collected in Tanford's Table 23-8 illustrates the extent of these effects (7). Molecular weight distribution is expected to have some effect as well -- Tompa (6) page 284. As with k', this multiplicity of factors affecting "a" seriously diminishes its value as a measure of branching.

(c) "g" Factor and Branching

In 1949, Zimm and Stockmayer (1) considered theoretically the effect that the kind and number of branches have on the dimensions of polymer molecules. Their results indicate that branching in a macromolecule reduces its average radius of gyration R_G (see G3) below that of the linear analogue of the same molecular weight. Comparison at the same number of segments allows the reduction in the radius of gyration of the branched species because of the increased segmental density around the center of gravity. Zimm and Stockmayer (1) then suggested that the ratio

$$\frac{R_G \text{ (branched)}}{R_G \text{ (linear)}}$$

which they designated as "g", should be a useful parameter in the characterization of branched polymers.

Flory and Fox (32) have predicted and shown experimentally that for linear molecules the following relation is valid

$$[\eta]_{linear} \propto \frac{(\sqrt{R_G})^3}{M} \qquad \ldots 8$$

and if it is assumed that the same proportionality holds for branched molecules, then equation 3, which applies to linear polymers, becomes for branched polymers

$$[\eta]_{branched} = K g^{3/2} M^{\frac{1}{2}} \alpha^3 \qquad \ldots 9$$

in which "g" essentially modifies linear chain theory to encompass the effect that branching has on the radius of gyration. Under theta conditions (G4), the expansion factor α becomes unity, and any difference in the polymer-solvent interaction for branched and linear polymers disappears. Then for branched and linear polymers of the same molecular weight, we obtain (combining equations 3 and 9)

$$\frac{[\eta]_{branched}}{[\eta]_{linear}} = g^{3/2} \qquad \ldots 10$$

$$g = \left(\frac{[\eta]_{branched}}{[\eta]_{linear}} \right)^{2/3} \qquad \ldots 11$$

Since "g" can be calculated for particular models, it was suggested that a combination of the theoretical tabulations of Zimm and Stockmayer (1) and the "g" values determined viscometrically should make it possible to characterize branched polymeric compounds.

The first test of this suggestion was made by Thurmond and Zimm (2) These authors calculated the distribution of the average number of crosslinks per polymer molecule for different fractions of a branched polymer and then, with the aid of the tables of Zimm and Stockmayer, obtained "g" values which were used to calculate the $[\eta]$ - $\bar{M}_w$ curve. Comparison of this calculated curve with the experimental one was made. Although there was qualitative agreement between the calculated and the experimental results, the quantitative agreement was only fair. The predicted effect of branching on the viscosity was considerably greater than was actually observed experimentally. On the basis of their experimental findings,

Thurmond and Zimm (2) questioned the validity of the assumption that the same proportionality exists between $[\eta]$ and $(\sqrt{R_G})^3/M$ for branched and linear polymer molecules. They further pointed out that molecular weight distribution must be taken into account: values of R_G and $\bar{M}_w$ obtained from light scattering measurements -- the usual procedure -- are Z-average and weight average values respectively (see G5). They concluded that the ratio of intrinsic viscosities $[\eta]_{branched}/[\eta]_{linear}$ at identical weight average molecular weights was probably the best method available for branch detection (since the averaging involved in the viscosity considerations is very close to a weight average) but urged the necessity for the development of a satisfactory theory of the intrinsic viscosity of branched molecules.

Stockmayer and Fixman (33), on the basis of the results of several experimental researches (34-36) (2), concluded that equation 10 overestimates the effect of branching on viscosity. Because "g" is calculated on the basis of the effect of branching on the reduction of the radius of gyration and then compared with an experimental "g" obtained from intrinsic viscosity ratios, these authors felt that the effective hydrodynamic radius (see G6) of a polymer molecule may be less sensitive to branching than its root-mean-square radius of gyration. With

this premise, they set out to find a correction factor for branching which would be more "accurately sensitive" to branching than $g^{3/2}$. Theoretical analysis of the effect of cruciform branching on the molecular friction coefficient (see G7) yielded the correction factor h (analogous to g), which is a function of the number of branches per branch point. They then equated

$$\frac{[\eta]_{branch}}{[\eta]_{linear}} = h^3 = \frac{\text{Effective hydrodynamic volume of branched molecule}}{\text{Effective hydrodynamic volume of linear molecule}} \quad ..12$$

which is similar to the Thurmond and Zimm equation but with h replacing $g^{1/2}$. The parameter h has numerical values such that the calculated values of the intrinsic viscosity of the branched polymer is in closer agreement with the experimental data, although it is still not perfect.

Application of the theoretical treatment of Stockmayer and Fixman (33) to the viscometric data of Thurmond and Zimm (2) gave, for the crosslinking index (see G8), a value of 0.61 as compared with the experimental value of 0.60 obtained by Thurmond and Zimm from a consideration of the molecular weights of the branched and linear polystyrenes. By contrast, Thurmond and Zimm (2), using the older Zimm-Stockmayer (1) treatment of $[\eta]_{branch}/[\eta]_{linear} = g^{3/2}$ had obtained a value of 0.40. This excellent agreement cannot, unfortunately, be taken as verification of the correctness of the

theoretical treatment of Stockmayer and Fixman, because, as Thurmond and Zimm recognized, the experimental value of the crosslinking index should have been 1.0. This value can be derived from the theory of polymerization kinetics which has been verified for a large number of systems (9).

More recently, in 1959, Zimm and Kilb (37) derived the equation

$$\frac{[\eta]_{\text{branched}}}{[\eta]_{\text{linear}}} = g^{1/2} \qquad \ldots 13$$

lending further support to the Stockmayer and Fixman thesis (33) that the relationship $[\eta]_{\text{branched}}/[\eta]_{\text{linear}} = g^{3/2}$ overestimates the effect of branching on viscosity. Recalculating the data of Thurmond and Zimm (2) with the aid of equation 13, Zimm and Kilb (37) obtained a cross-linking index of 0.77, which is twice the value obtained in the original work and which is larger than the value of Stockmayer and Fixman, 0.61, (33) but which still is less than the kinetically expected value of 1.0. The calculated dependence of $[\eta]$ on M for the branched compound also appears to agree fairly well with the experimental points up to a molecular weight of two million, the highest molecular weight studied. Similar calculations on the data obtained by Schaefgen and Flory (35) with tetra and octa chain (see G9) polymers gave better agreement with experimental $[\eta]$ - M_w

curves than did calculations involving equation 10.

Kilb (38) continued the work of Zimm and Kilb (37), extending it to polydisperse systems. After considerations of the possible physical properties that might permit the detection and estimation of branching, he concluded that the most practicable method of branch estimation is still a consideration of the ratio of the intrinsic viscosity of branched to linear species at identical weight average molecular weight -- provided that the ratio is equated to $g^{1/2}$.

Some of the difficulty in employing equation 10 may lie in the fact that branched systems have hydrodynamic interactions that are not entirely dependent on particle size. Bueche (39) has also taken the viewpoint that the intrinsic viscosity is not simply proportional to the radius of gyration for branched polymers as it happens to be for linear ones. This, in essence, supports what Stockmayer and Fixman suspected -- the radius of gyration of a branched polymer is not a good measure of its hydrodynamic volume. Despite lack of extensive experimental evidence to support these views at present, the few studies involved with this point indicate that the suspicions are not unfounded. Arond and Frank (40) discovered that for a series of fractions of native dextrans the proportionality constant ϕ' of Flory's theory varied with molecular weight and

suggested branching as one of the possible sources for this variability. This, in turn, means that the assumptions in deriving equation 10 were incorrect. In their study of branched dextrans, Senti et al (3) found, after corrections for polydispersity had been made, that the value obtained for the Flory constant was greater than the accepted value for linear molecules by a factor of 1.8. There is some contention that "g" only detects certain kinds of branching. Billmeyer (41) claims that only very long branches cause a reduction in the intrinsic viscosity of branched polymer samples. Trementozzi (4) has presented evidence that long chain branching increases with molecular weight. All this evidence suggests that the curvature (see Figures 1 and 2) in the $\log[\eta]$ - $\log M_w$ plot is due primarily to the long chains and that the g or h correction factors measure only long chain branching.

It is evident, then, that before precise knowledge of branching in a polymer can be obtained from experimental measurements, there must be further development of the theory of the viscosity of solutions of branched polymers. The attempt to adapt a theory worked out for linear polymers, to the more complicated branched molecules, has apparently failed.

To sum up, then, it now appears that the exponent "a" of the Mark-Houwink equation can only give a rough indication of the occurrence of branching in the polymer. The interaction parameter k' of the Huggins equation has been shown by empirical correlation and theoretical calculations to be dependent upon branching in addition to being dependent upon several other factors. It can be used, tentatively, as an indicator of "bushy" branching. Long chain branching can be handled semi-quantitatively with the tabulated g factors of Zimm and Stockmayer (1) and the ratio of the intrinsic viscosities of branched and linear polymers at identical molecular weight only if this ratio is equated to h^3 or $g^{1/2}$.

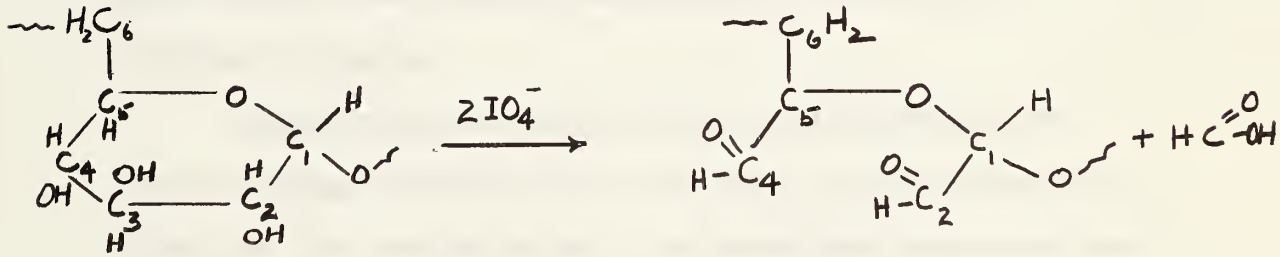
III. Dextran

The polymer used in this investigation was dextran, a polysaccharide which could be obtained readily and as a fairly well defined linear or branched polymer.

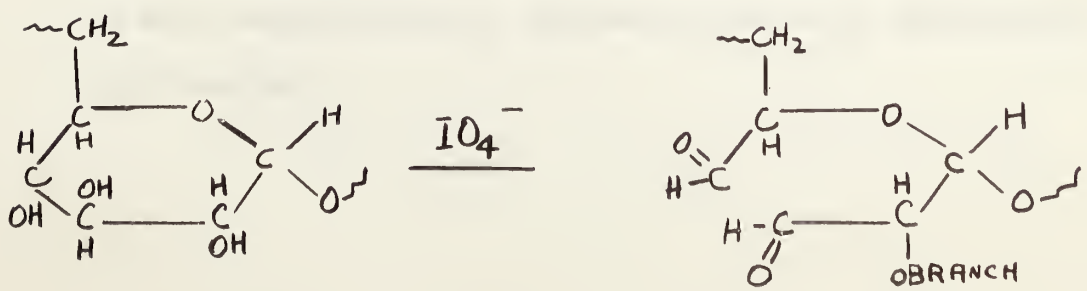
Dextran is a collective term for a group of polysaccharides grown on a sucrose substrate which are composed of anhydroglucose residues predominantly linked 1,6. Because of the extensive use of it in solution as a plasma substitute (42 - 44), much effort has been expended on determining the fine structure of dextran. It is now well known that dextrans differ from sample to sample, depending upon the particular

bacterial strain producing them (45). For details, extensive reviews (46 - 48) or original research papers (45) (49 - 58) may be consulted. A brief outline of methods used in the determination of the details of the fine structure of dextran and of the proposed structures themselves, is included here. (The chemical techniques outlined are familiar in polysaccharide chemistry.)

The earliest work on dextran fine structure involved oxidation of the polysaccharide with periodic acid or its salts. Anhydroglucose units linked 1,6 have three neighboring hydroxyl groups. Periodate oxidation of this arrangement yields two aldehyde groups and formic acid as follows:



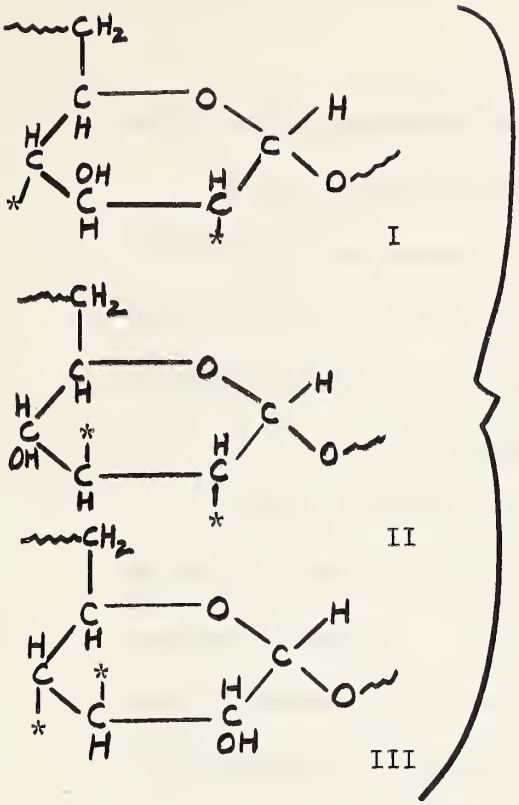
If there is an additional linkage at either the 2 or 4 position, no formic acid is produced and only the dialdehyde is obtained as product:



Existence of an additional linkage to that of the regular 1,6 at the 3 position does not allow any oxidative attack of the anhydroglucose unit. Branching at 2 and 3, 3 and 4, or 2 and 4 also renders the anhydroglucose unit inert to oxidation. Hence, from a consideration of formic acid produced, initial periodate and final periodate concentrations, and the amount of dextran used, the percentages of 1,6-like, 1,3-like and 1,4-like linkages can be calculated.

It is unfortunate that the periodate method does not differentiate between single branching at position 3 and the double branching possibilities enumerated. If only estimates of the types of linkage are required, the disadvantage is somewhat offset by the relative convenience of carrying out a periodate oxidation.

A method which does allow differentiation between the various linkage possibilities is one which involves methylation, hydrolysis and identification of the methylated monosaccharides. Since every free hydroxyl is made into the methyl ether, a greater degree of discrimination is introduced into the determination of linkages of the anhydroglucose unit by the methylation technique than is present in the periodate technique, in which several different combinations cannot be distinguished. To illustrate:



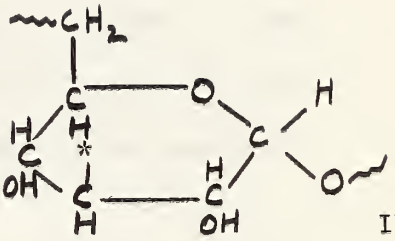
I

II

III

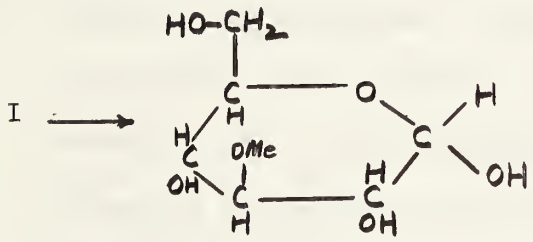
* branch

are not distinguishable from

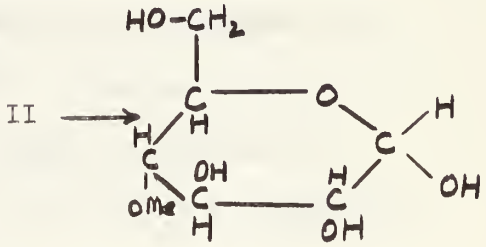


IV

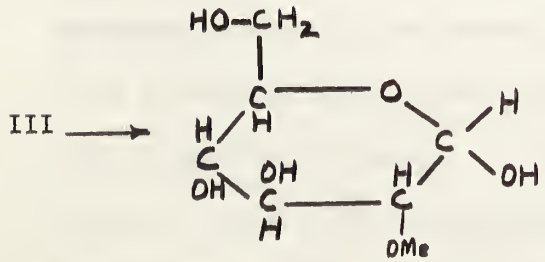
by periodate oxidation. However, with the methylation technique one obtains



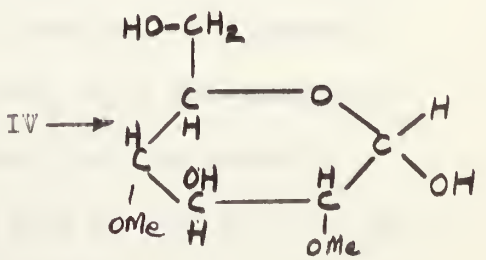
I



II



III



IV

which can be separated by gas-liquid chromatography.

Not only are the types of linkages contained in dextran of interest, but also the lengths and types of branches. From the studies on limited hydrolysis of Jeanes (47) and Jones (54), it would appear that as many as eighty percent of the branches are only one or two glucose units long.

Physical studies, particularly the viscosity work of Wales (59) and Senti (3), are in essential agreement with the chemical evidence. The viscosity studies could not, of course, give information on the type of linkage, but do give information on the general structural features.

Other types of physical studies have been carried out with infrared absorption (56) and optical rotation (45) (60). The importance of both of these techniques has been in the detection and estimation of the number of 1,3 linkages after correlation with the chemical methods. The one optical rotation study of the cuprammonium complex of dextran (61) allowed an estimation of the amount of 2,3 and 3,4 neighboring hydroxyl entities.

The evidence gathered by these techniques leads to the following picture of the structure of dextran. It appears to be a polymer with its main chain made up of anhydroglucose units joined by 1,6 bonds. Branches which are generally only one or two units long originate from some of the 2, 3 and 4 positions. When longer branches occur they may, themselves,

be branched.

IV. Polyelectrolytes

While a considerable number of reviews concerned with the viscosity behaviour of neutral polymers have appeared (see Section B I), virtually none is available concerning the different viscosity behaviour of polyelectrolytes (62). Therefore, a brief discussion of the experimental reduced viscosity plots obtainable in aqueous solution seems in order.

Whereas graphs of η_{sp}/c vs c for neutral polymers are generally straight lines at moderate concentrations (1% or less) in pure solvent, those for polyelectrolytes in pure solvent rise monotonically with dilution (while other effects appear at extremely low concentrations (10^{-5} g./ml.) -- these are not relevant to the present discussion).

There are two ways in which rectilinear behaviour can be obtained. One is the use of solvents consisting of highly concentrated solutions of simple electrolytes. The second is the method known as isoionic dilution. In solvents consisting of dilute solutions of simple electrolytes, the reduced viscosity, η_{sp}/c, rises and then decreases, causing a maximum to appear in the η_{sp}/c vs c plot.

Staudinger (63) was the first to demonstrate with a well defined polymeric electrolyte (polyacrylic acid) the monotonic rise of the η_{sp}/c vs c curves. Fuoss (64) showed that this

rise in reduced viscosity was characteristic of polyelectrolytes in pure solvents. What has become a classic example is reproduced in Figure 3. Similar behaviour has been demonstrated by Winter and Beckman (65) for a series of carboxymethyl polysaccharides which had been substituted to different degrees. Polystyrene sulfonic acid reduced viscosity-concentration plots obtained by Kato, Nakagawa and Akamatu (66) were also of identical form. The technique of isoionic dilution was first suggested by Pals and Hermans (67), who demonstrated straight line viscosity-concentration relationships with sodium carboxymethyl cellulose and sodium pectinate. These authors argued that if the ionic strength of the polymer solution system were kept constant as the polymer solution was diluted, there would be no expansion of the polymer coil and hence no increase in η_{sp}/c on dilution. Figure 4 illustrates the type of curve obtained. This type of behaviour has also been observed in the studies of Terayama and Wall (68) on potassium cellulose sulfate and by Rezanowich and Goring (69) on lignin.

Fuoss (64) found that if a small amount of simple electrolyte ($\ll$N/100) were added to the water which was being used as the solvent for his polyelectrolytes, curves such as exhibited in Figure 5 were obtained. To illustrate the phenomenon with as great a number of curves as possible, the results of a later

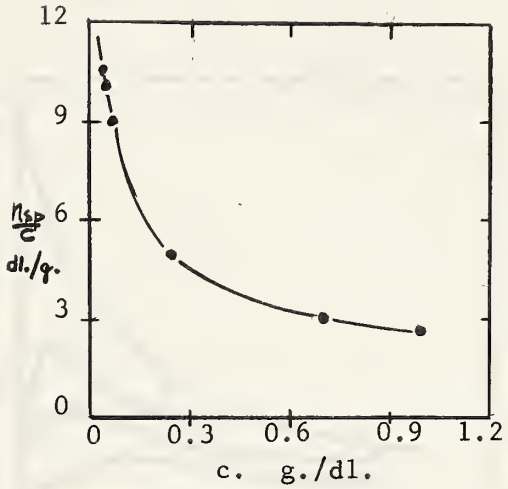


Figure 3. Plot of reduced viscosity vs concentration: poly(N-n-butyl-4 vinyl pyridinium) bromide in water.

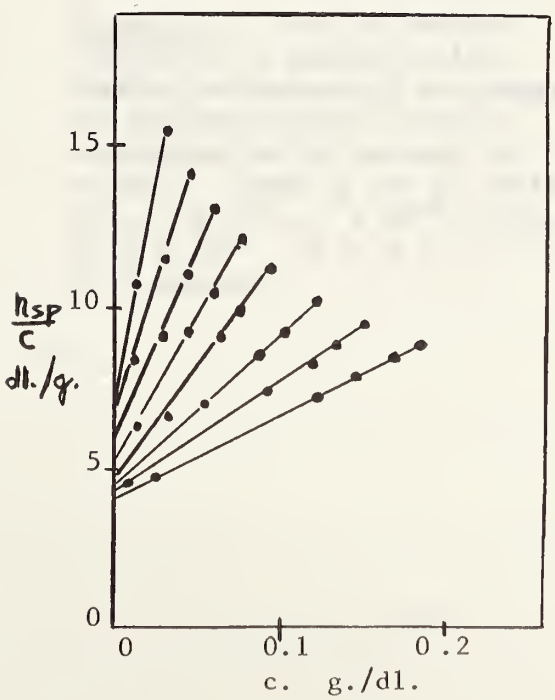


Figure 4. Plots of reduced viscosity vs concentration (isoionic dilution): sodium carboxymethyl cellulose in aqueous sodium chloride, reading top to bottom: ionic strengths of 1.00×10^{-3}, 1.50×10^{-3}, 2.50×10^{-3}, 3.10×10^{-3}, 3.50×10^{-3}, 4.10×10^{-3}, 5.00×10^{-3} and 6.00×10^{-3} moles/l.

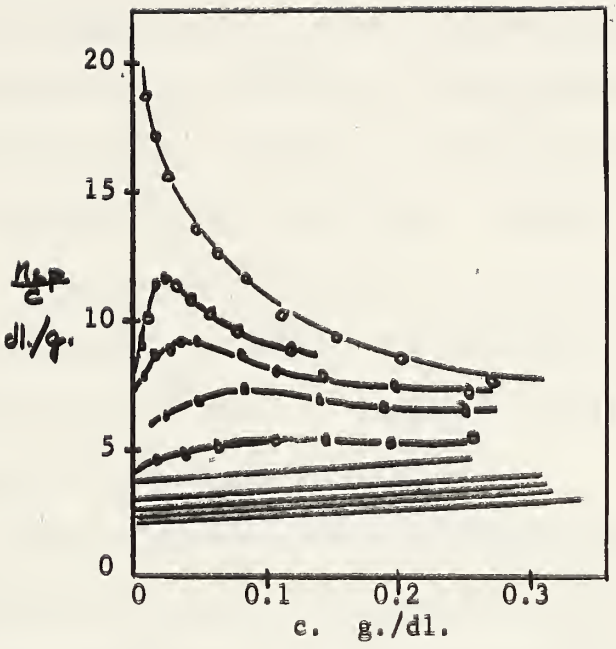


Figure 5. Plot of reduced viscosity vs concentration: sodium carboxymethyl cellulose in sodium chloride solution, concentration of solvent in moles/l. (reading top to bottom): nil, 5×10^{-4}, 1×10^{-3}, 2.01×10^{-2}, 2.51×10^{-2}, 5.02×10^{-2}, 0.10 and 0.20 moles/l.

study (68) have been presented. It is apparent that the higher the concentration of simple electrolyte, the less pronounced the maximum, until at about N/100 the maximum disappears altogether and rectilinear relationships are obtained.

Polyelectrolytes, of course, are polymers and their viscometric behaviour reflects that fact. However, three different effects, all arising from the presence in the molecules of charged groups, are generally recognized as contributing significantly to the viscometric behaviour. These are called the first, second and third electroviscous effects.

The first electroviscous effect, which is due to the interaction between the charged polyelectrolyte particle and its accompanying counter-ion cloud, arises because the molecule and its counter-ions straddle the velocity gradient of the flowing liquid. This causes the polymeric particle to move at a greater or lesser rate than the counter-ion cloud relative to the liquid. Electrostatic interaction above that which exists under stationary equilibrium conditions arises, causing an extra expenditure of energy. This effect, however, contributes negligibly to the viscosity of polymeric electrolytes and large-size colloids.

The second electroviscous effect has been attributed to particle-particle interaction. In neutral polymer solutions, particle-particle interaction takes place because of hydrodynamic and mechanical encounters, with each encounter contri-

buting to the viscosity because of an extra expenditure of energy. In aqueous solutions of low concentration (< 0.1 g./dl.) it is found experimentally that the slope of the η_{sp}/c vs c curve is proportional to $[\eta]^2$ (70), indicating that the initial rise of the curve is due primarily to particle-particle interaction. Similar results were reported by Pals and Hermans (67).

Fuoss (71) (72) postulated that with polyelectrolytes there exists a counterion mass action effect upon dilution with water or very dilute simple salt solutions and that this causes an increase in the polymer particle volume because of the increase in electrostatic repulsion as more charge develops on the polymer molecule. This effect is called the third electroviscous effect. The several attempts made (73 - 76) to calculate the dimensions of polymer molecules expanded by electrostatic repulsions have indicated that substantial expansion is to be expected.

Experimentally, expansion was demonstrated by Katchalsky (77), Rice and Harris (78), Fuoss (79), Arnold and Overbeek (80), Markowitz and Kimball (81), and others (82 - 86). Conway (70) has interpreted the reduced viscosity plot of polymethacrylic acid in water by invoking the second and third electroviscous effects -- the second effect operating in the most dilute regions while the third operates in the

high concentration regions beyond the maximum. Similar views were expressed by Mathieson and Porter (87). More directly, Doty (88) (89) has shown by light scattering measurements that the volume of a polyelectrolyte molecule in solution can change appreciably, when diluted.

C. EXPERIMENTAL

I. Introduction

The possibility of branch detection in the high molecular weight region depends primarily on the fact that a branched polymer molecule pervades a smaller volume in solution and hence has a lower intrinsic viscosity than its linear analogue of identical molecular weight (Section B II c). Branched and linear species of the same low molecular weight do not exhibit a significant difference in intrinsic viscosity, and therefore the volumes pervaded by the two macromolecules must be essentially the same. Both are believed to assume a random coil configuration in solution. Hence one may represent them thus:

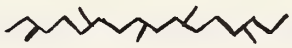
linear

branched

The problem arises, therefore, that, if we are to use the viscometric techniques on low molecular weight samples, some modification must be made to the hydrodynamic volumes of the molecules themselves. This would be done with the hope that, if the volumes themselves are modified sufficiently, the difference between them will be magnified to the point where it is easily measurable. For example, if the linear

and branched molecules in the figure could be completely extended in solution, the branched would be shorter than the linear one

linear

branched 

and the intrinsic viscosity (volume pervaded) of the branched molecule would be measurably smaller than the linear sample. Under these idealized conditions, the intrinsic viscosities (or the reduced viscosities) could be used to detect and estimate branching. Unfortunately, it is impossible to realize this completely extended state of the polymer molecule, even in the available "best" solvents.

Expansion of the polymer molecule beyond that achieved in "good" solvents can be accomplished by introducing like charges on the segments of the polymer chain, and so causing them to repel one another strongly. This could be done by converting the polymer into a polyelectrolyte. In order to test our hypothesis that a difference does exist between the viscosity behaviour of linear and branched dextrans in the low molecular weight regions , it was decided to convert linear and branched low molecular weight dextrans into polyelectrolytes and compare the viscosity behaviour of the linear and branched charged polymer.

The detection and estimation of branching discussed in Sections A and B II c depends on differences in intrinsic viscosities between linear and branched polymers. However, the usual method of obtaining the intrinsic viscosity for polymers, involving rectilinear extrapolation to zero concentration of the reduced viscosity-concentration plot, is not at all satisfactory when applied to polyelectrolytes, because the η_{sp}/c vs c curves are not usually straight lines. When the solvent is a relatively concentrated salt solution (see Figure 5), the curves are straight, but then the polyelectrolyte expansion effect has been practically swamped out. Thus the advantage sought by the introduction of charged groups is completely (or nearly completely) vitiated by the concentration of counter ions.

The isoionic dilution method (67) also gives linear viccosity concentration curves (see Figure 4), but this method too has disadvantages. If it is used in solvents of high ionic strength, it has the same failing as the high constant salt dilution method previously discussed. If it is used in solvents with low ionic strength, the slope of the viscosity concentration curve is too steep to permit a reliable extrapolation. Besides that, the polymer concentrations required to give linear data are so low as to reduce considerably the precision of the viscometric measurements.

Clearly, then, a comparison of the intrinsic viscosities of our branched and linear polyelectrolytes would not be as simple and useful as we had hoped. Fortunately, however, the reduced viscosity η_{sp}/c is likewise a measure of the volume of the macromolecule in solution, and by comparing reduced viscosities at the same concentration one can compare the volumes of the molecules of the polyelectrolytes under conditions where they are highly expanded by mutual repulsion of their charges. For this reason it was decided to compare reduced viscosities measured in an aqueous solvent in which the simple salt concentration was very low ($<<$ N/100). The type of viscosity curve to be expected is depicted in Figure 5. It was also decided that since hydrodynamic volumes are of prime interest, it would be better to compare linear and branched polyelectrolytes derived from parent materials which have identical intrinsic viscosities rather than identical molecular weights.

Therefore, on the assumption that conversion of low molecular weight linear and branched macromolecules to polyelectrolytes would produce significant differences in their viscosity behaviours, the viscometric study of linear and branched dextran polyelectrolytes was undertaken.

II. Materials

The branched dextran sample, NRRL B-742, was obtained from Dr. Allene Jeanes, Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. Dr. Jeanes' designation for this sample was "Clinical size fraction B-2-b, P.P.31". It had the following properties:

Jeanes' values: $\bar{M}_w$ 6.1×10^4 (determined by light scattering)

$[\eta]$ 0.153 dl./g.

% 1,6 linkage: 70

% 1,4-like linkage: 13

% 1,3-like linkage: 18

Our values: $[\eta]$ 0.157 dl./g.

% 1,6 linkage: 70

$s_{20,w}(1\%) = 2.56$ Svedbergs

This material was used without further purification or fractionation.

The sample of linear dextran was obtained through the courtesy of Dr. A.F. Charles, Connaught Medical Research Laboratory, Spadina Crescent, University of Toronto, Toronto 4, Ontario, in the form of a clinical preparation (a solution which was 6% dextran and 0.9% NaCl). This dextran sample was recovered as described in Section C III a. Some of the physical properties of this linear dextran were:

Our values: $[\eta]$ 0.164 dl./g.

% 1,6 linkage: 95

$\bar{M}_w$ 3.9×10^4 calculated from the measured sedimentation coefficient using the s-$\bar{M}_w$ relationship of Senti (3)

$s_{20,w}(1\%) = 2.09$ Svedbergs

Isomaltotriose was obtained pure from Dr. D.H. Hutson, Royal Hollaway College, London, England.

Dextran sulfates and carboxymethyl dextrans were prepared according to the procedures outlined in Sections C III c and C III d.

Demineralized water was prepared using a Crystalab water demineralizer and contained 0.2 - 0.4 p.p.m. NaCl, and was routinely used for all solutions.

Chlorosulfonic Acid, Practical (Eastman Kodak Company, Rochester, N.Y., U.S.A.) was redistilled and a large middle fraction, which distilled at 147°C, was collected and used in all sulfations.

Other chemicals used without further purification were:

Pyridine (Fisher Scientific Company, Toronto, Canada) certified reagent

Formamide (Matheson Coleman and Bell, East Rutherford, N.J.) practical

Monochloroacetic acid (Fisher Scientific Company, Toronto, Canada) certified reagent grade

Potassium hydroxide (Mallinckrodt Chemical Works, Montreal, Canada) analytical reagent

Potassium chloride (Mallinckrodt Chemical Works, Montreal, Canada) analytical reagent

Methanol (Mallinckrodt Chemical Works, Montreal, Canada) anhydrous, analytical reagent

Deuterium oxide (Merck, Sharpe and Dohme) minimum isotopic purity 99.7 atom percent deuterium.

III. Techniques

(a) Isolation and Fractionation of Linear Dextran

Samples of the clinical preparation of linear dextran, which contained 0.9% sodium chloride, were dialyzed against demineralized distilled water before use. Prior to each change of water, the water outside the bag was tested with $AgNO_3$ for chloride ion. Dialysis was continued for several changes beyond that at which chloride could no longer be detected. An aliquot of the dialyzate was freeze-dried and the intrinsic viscosity of the dextran determined: it was 0.261 dl./g.

In order to obtain a sample with an intrinsic viscosity close to that of the B-742 dextran ($[\eta] = 0.157$), the dextran was fractionated in the following manner. The 6% dialyzed dextran solution was diluted to approximately 1%. Methanol was added in small increments to this solution until a faint turbidity developed, after which the turbid liquid system was allowed to stand at

room temperature for five or six hours. The mixture was centrifuged in a refrigerated Spinco preparative centrifuge, employing a Spinco constant flow device. The rotor was pre-cooled to about 10°C below room temperature. The temperature of the centrifuge well was set at room temperature and the centrifuge was brought up to speed (15,000 r.p.m. with no liquid flowing) in about 15 minutes. The turbid liquid system was allowed to flow in and the temperature of the outflowing supernatant was recorded. It did not vary at any time by more than ~~two or~~ three centigrade degrees from the temperature of the inflowing liquid (room temperature). This procedure minimized change in the conditions of the fractionation equilibrium. An aliquot of the supernatant was freeze-dried and the intrinsic viscosity of the isolated fraction determined.

The whole procedure was repeated until the supernatant liquid yielded a sample of the desired intrinsic viscosity. The large volume of the supernatant liquid was then reduced in a rotary flash evaporator, and the dextran was finally recovered by freeze-drying.

(b) Dialysis

Seamless cellulose dialysis tubing, of 1 1/8"

diameter when inflated, obtained from Fisher Scientific Co., was used throughout. Solutions were dialyzed against running cold tap water ($\approx 18^{o}C$) for several days and then against demineralized distilled water (4 liters). The water was changed six to ten times, as required.

(c) Preparation of Dextran Sulfates

In the standard method of preparing the inorganic esters of carbohydrates, pyridine is used as the reaction medium and chlorosulfonic acid as the sulfating agent. The reaction is generally carried out at temperatures of about $70^{o}C$.

There are several disadvantages to the application of the standard procedure to our dextran samples. At such a high temperature and under such harsh conditions, extensive branch hydrolysis and chain scission would be probable. Such degradation would be most undesirable in this study. Moreover, the solubility of dextran and dextran sulfate in pyridine, even at the "standard" reaction temperature, is low. As a result, the sulfation reaction is a heterogeneous one and the product is not as uniformly modified as is desirable.

In an attempt to avoid the disadvantages of sulfation in a pyridine medium, the dextran sulfations were

carried out in formamide, in which both dextran and dextran sulfate are soluble. To formamide (70-80 mls.) at 0°C, purified cold chlorosulfonic acid (5-10 mls.) was slowly added dropwise from a buret fitted with a teflon stopcock. After the addition was complete, 1 gram of dextran was added in solution in formamide (1 gram in 30 mls.). This solution had been prepared at room temperature and then cooled to 0°C. The reaction mixture was allowed to react at 0°C for up to six hours, depending on the degree of sulfation desired. Potassium hydroxide pellets were then added to the solution slowly, keeping it at 0°C, until an aqueous solution of a small aliquot of the solution was basic to litmus. The solution was then dialyzed against running tap water and after several days, when the solution no longer turned red litmus blue, the dialysis was continued against several changes of demineralized water. To insure that every ionogenic center of the dextran sulfate carried a potassium cation, the dialyzate was passed through a Dowex 50 cation exchange resin which had been properly charged with potassium ion. The polyelectrolyte was filtered through a sintered glass filter and freeze-dried. (The acid derivative was not isolated, as this form of dextran sulfate is unstable.)

(d) Preparation of Carboxymethyl Dextran

Preparation of the carboxymethyl dextran was carried out essentially as described by Winter and Beckman (65). Minor modifications were as follows. The monochloroacetic acid, rather than being added directly to a solution of dextran in 20% W/W NaOH at room temperature, was added, in three times the stoichiometric amount, to 80 mls. of 20% NaOH at 0°C, and then 30 mls. of cold 20% NaOH-dextran solution (containing 1 gram of dextran) was added. Reaction times were one, two, three or four days at 0°C for the low-degree-of-substitution series (less than 0.21 carboxymethyl groups/anhydroglucose unit). The two samples substituted to a high degree (more than 1.0 carboxymethyl group/anhydroglucose unit) were obtained by a two-stage carboxymethylation at 18°C. Isolation of the derivative after the first stage of carboxymethylation was effected before the second stage was started. Each reaction was carried out for about thirty hours and after the second etherification the reaction mixture was treated in the same manner as products of the reaction at 0°C (dialysis, ion exchange, etc.). Double carboxymethylation was required to obtain a carboxymethyl dextran whose degree of substitution was comparable to that of the sulfates. Most of the material was isolated as the salt,

but a small amount was converted to the acid form by ion exchange and isolated by freeze-drying.

(e) Analytical Procedures

Since the potassium dextran sulfates and the potassium carboxymethyl dextrans contain non-volatile inorganic residues, these compounds were analyzed by igniting, to constant weight; the potassium dextran sulfate to potassium sulfate and the potassium carboxymethyl dextran to potassium oxide. After each ignition, the hot crucibles were put into desiccators over phosphorous pentoxide. After cooling there was always a slight vacuum in the desiccators, indicating a negligible probability of moisture absorption by the inorganic residues as the crucibles cooled. When cool, the crucibles were quickly weighed on a sensitive Mettler balance. In calculating the sulfate content of the dextran sulfates, the number of moles of K_2SO_4 remaining in the crucible must be doubled since, for each mole of sulfate substituent converted to K_2SO_4 , one mole of sulfate substituent is volatilized as H_2SO_4.

The two carboxymethyl dextrans with high degree of substitution (> 1.0 carboxymethyl group/anhydroglucose unit) and the carboxymethyl dextrans used in Section D V c were analyzed by titrating the acid form with sodium

hydroxide solution to a phenolphthalein end point under purified nitrogen. These samples were analyzed by both direct and back titration. The back titration involved adding an excess of base to the carboxymethyl dextran solution, allowing it to stand corked for about ten hours, and then titrating the excess sodium hydroxide. Identical results were obtained by the two titration methods. See Table I for analytical results.

(f) Proton Magnetic Resonance Spectroscopy (PMR)

A Varian A60 Proton Magnetic Resonance Spectrometer, operating at 60 Mc/s, was used to obtain the proton resonance spectra.

All sulfate derivatives used in the PMR study were converted to the more soluble sodium salt. In all samples the labile protons of the hydroxyl groups were exchanged for deuterons by a three-times repeated process of dissolving in deuterium oxide and freeze-drying. All spectra were measured with D_2O as solvent.

Data for plots of peak areas against concentration were obtained in the following manner. 0.5 mls. of a stock solution (0.25 g./ml.) was made up in a small vial fitted with a rubber cap. The smallest amount of solution needed for a PMR run (0.4 ml.) was transferred with a dry syringe to the PMR sample tube, the latter was corked,

and the spectrum of the sample was determined. Next, 0.1 or 0.2 mls. of D_2O were added, the contents were thoroughly mixed in the sample tube, and the spectrum of the diluted solution was determined. Six or seven dilutions were carried out in this way, and a spectrum was measured for each solution.

The external standard was tetramethyl silane.

The peak areas were measured carefully with a planimeter.

The linear and branched sodium dextran sulfates used in the PMR study analyzed 9.96% and 8.27% sulfur, respectively.

(g) Infrared Spectroscopy

Spectra were obtained on a Perkin-Elmer 221 double-beam recording spectrophotometer. Samples were prepared for the spectrophotometer by the KBr pressed-disc technique (90). The ratio of sample to potassium bromide was generally 2 mgs. to 300 mgs. After preliminary grinding with pestle and mortar, the mixture was ground in a ball mill for one hour. The charge put into the pellet die was 300 mgs. of mixture. A standard (28,000 lbs.) hydraulic pressure was used for all pellet preparations. Several die charges were prepared by freeze-drying dissolved potassium bromide and sample. This technique

yielded no better results than the dry grinding procedure.

Direct infrared detection of branching after sulfation is not possible since the substituted sulfate causes a peak to appear at 810 $cm.^{-1}$ which completely masks the 790 $cm.^{-1}$ peak which is attributed to 1,3 branching (45) (56) and which is the sole basis of the spectroscopic detection of branching in dextran. An attempt was made to use difference spectroscopy. In this procedure, one tries to cancel the extraneous peak by placing an equal amount of the chromophore responsible for it in the second beam of the spectrophotometer. This involved producing two sets of two pellets each: one set (linear dextran and branched dextran) in which each contains the same weight of dextran, and potassium bromide, and the other set (linear dextran sulfate and branched dextran sulfate) in which each pellet contains the same weight of dextrans and potassium bromide as in each of the first set and the same weight of sulfate residue.

In linear dextran, the glucosidic linkages will all be 1,6. In the same weight of a branched dextran, there will be the same number of glucosidic linkages, but of different kinds -- 1,6; 1,3; etc.

The 1,3 linkage manifests itself as a peak in the infrared spectrum at 790 $cm.^{-1}$, and the 1,6 linkage as a

peak at 766 cm.$^{-1}$. If two pellets are made up, one containing linear dextran and the other the same amount of branched dextran, and if these are examined in a double beam infrared spectrophotometer with the branched dextran in the working beam, the 1,3 signal will be recorded in the normal way -- below the base line -- for the sample in the working beam has a greater number of these linkages. The 766 cm.$^{-1}$ signal is recorded as being negative owing to the greater concentration of 1,6 linkages in the reference beam. The difference between the extremes of absorption can be taken as a measure of branching. (Since two samples, one linear and the other branched, of equal degree of sulfate substitution were available, the problem of matching and cancelling the sulfate peak was not difficult.)

Difference spectra were determined in this way for both the unsubstituted dextrans and their dextran sulfates. A comparison of the difference spectra in the region 800 cm.$^{-1}$ to 760 cm.$^{-1}$ was then used to obtain an indication of the extent of branch hydrolysis.

The difference spectra are shown in Figure 6.

The carboxymethyl group does not absorb infrared radiation in the vicinity of 790 cm.$^{-1}$. Therefore, one can make a direct comparison of the amount of branching in carboxy-

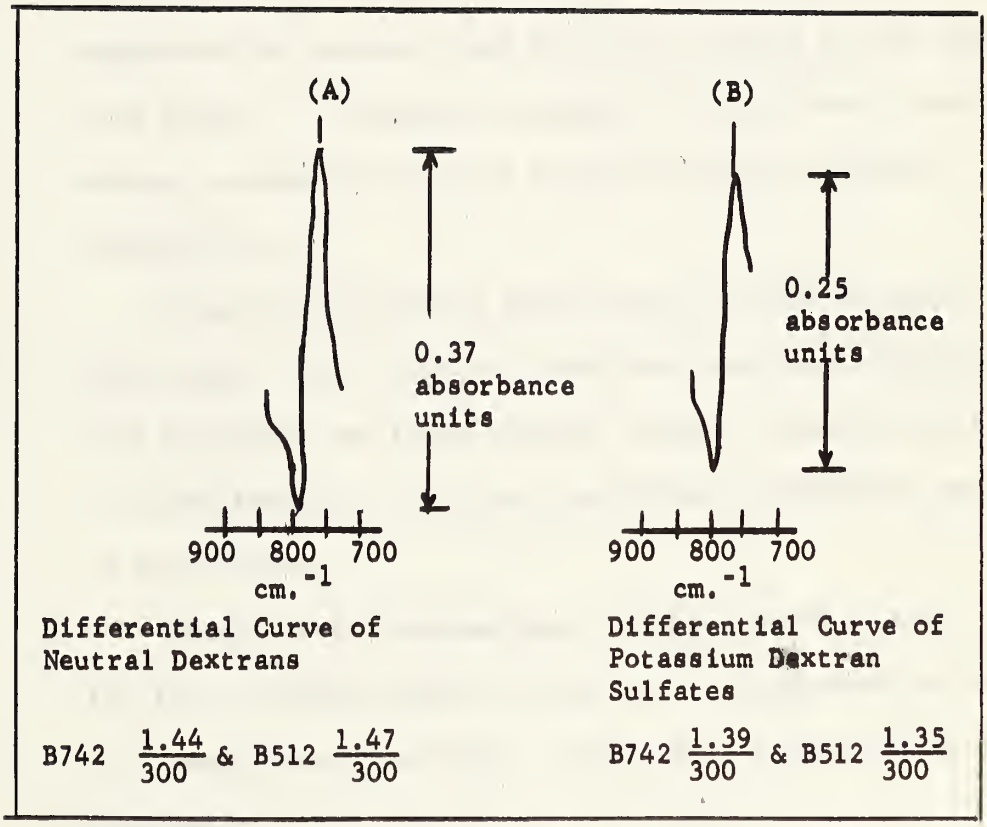


Figure 6. Differential infrared spectra.
(A) linear and branched dextran
(B) linear and branched dextran sulfates (9%S)
Ratios are weight ratios of dextran to KBr in the pellets (see Section C III g).

methyl dextrans with that in the unsubstituted dextrans (see Figure 7).

(h) Ultracentrifugation

The instrument used was a Spinco Model E ultracentrifuge, equipped with schlieren optics. Conventional sector-shaped cells were used in the determination of sedimentation constants and molecular weights by the Archibald Method. Synthetic boundary cells (91) were used to obtain concentration values required for the Archibald calculations.

Kodak metallographic plates were used in the ultracentrifuge; after exposure, they were developed with Kodak D-76 developer for three minutes, washed, fixed for half to three-quarters of an hour, and finally washed for two to three hours.

Photographic diagrams were transferred to 10 x 10 (to the cm.) graph paper by tracing an enlargement projected at a magnification of 10x. Areas were measured with a planimeter.

Densities (required for the calculation of partial specific volumes) were determined with 25 ml. pycnometers when enough solution was available. Otherwise, 2 ml. volumes of solution were made up and the densities were

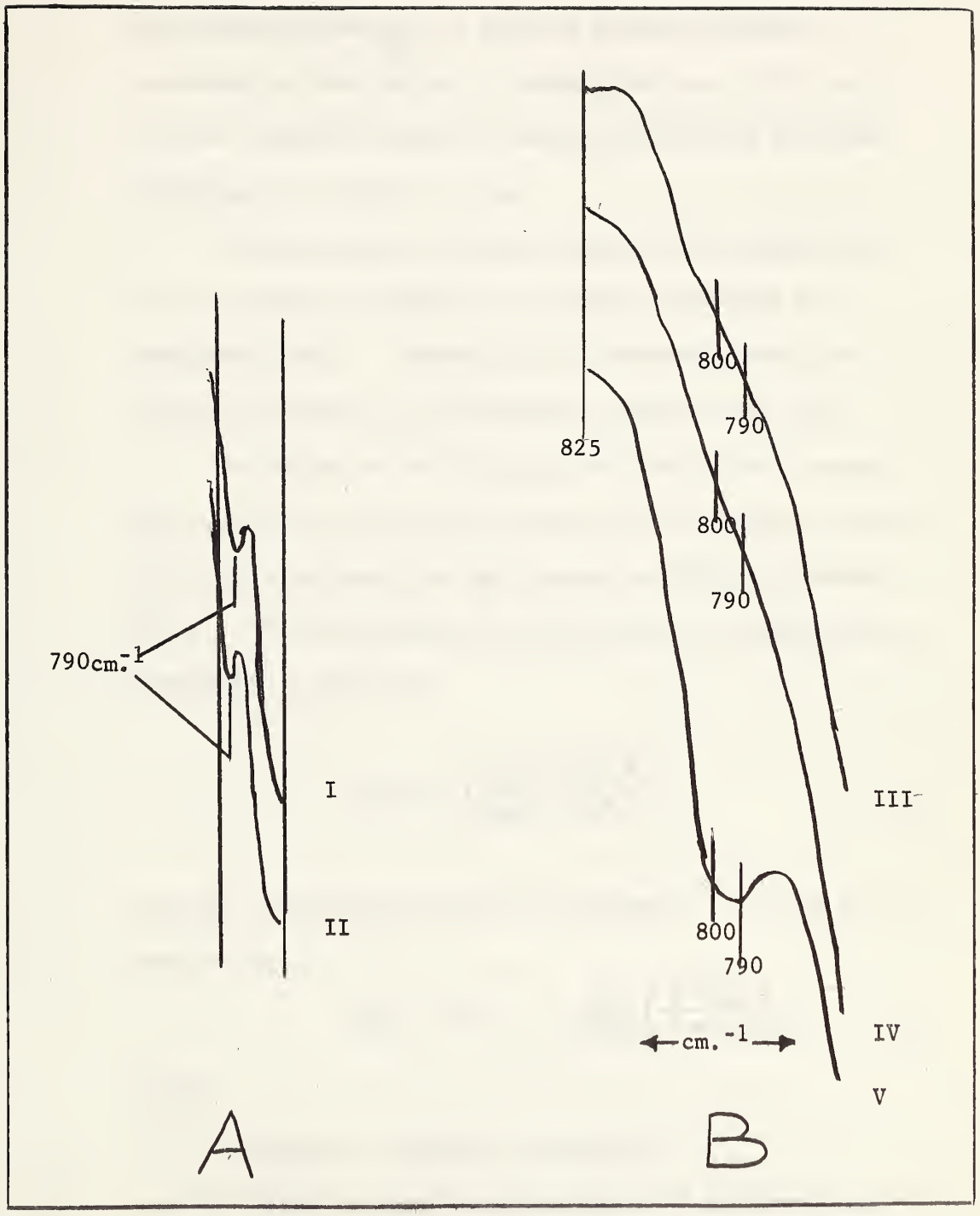


Figure 7. Infrared spectra of linear and branched dextrans and potassium carboxymethyl dextrans (CMD).

A. I branched dextran
II branched CMD
(spectra expanded 5 x ordinate)

B. III linear dextran
IV linear CMD
V branched CMD
(spectra expanded 5 x ordinate, 4 x abscissa)

determined by means of a density gradient column, according to the method of Linderstrøm-Lang (92). The partial specific volume of dextran calculated from the densities was 0.650 c.c./gm.

Ultracentrifuge molecular weights were determined by the Archibald method in the manner described by Schachman (93). Sedimentation constants were also obtained according to Schachman's instructions (93).

The method of calculating the distribution curves was essentially that used by Gupta, Robertson and Goring (94) and also described by Claesson and Moring-Claesson (95). The distribution function g*(s) of sedimentation constants is given by

$$g^*(s) = \left(\frac{dc}{dx}\right) \frac{\omega^2 t x^3}{C_o X_o^2} \qquad \ldots 14$$

and the sedimentation velocity constants of the i th species by

$$S_i = S_m + \frac{2}{\omega^2 t}\left(\frac{x - x_m}{x + x_m}\right) \qquad \ldots 15$$

where

ω angular velocity (radians/sec.)

t time in secs. from the start of sedimentation

x distance in cm. of a point in the boundary from the axis of rotation

x_o distance in cm. of the meniscus from the axis of rotation

x_m distance in cm. of the maximum value of dc/dx from the center of the rotor

c_o concentration of original solution (g./dl.)

s_m sedimentation constant of the species at x_m

Generally, equation 14 must be corrected (a) for diffusion effects, by evaluating g*(s) at infinite time (ie. g(s)) where diffusion is negligible by extrapolation to 1/t = 0 of a plot of g*(s) vs 1/t and (b) for concentration effects by extrapolating a plot of g(s) vs c to c = 0. Figure 8 shows how the distribution function g*(s) varies with time. Because the calculations involved in the treatment of ultracentrifuge data are very lengthy, several shortcuts were taken which considerably decreased the time required for obtaining the desired results. In Figure 9 are plotted, for comparison, the apparent distribution of s_1 from photos taken forty and forty-eight minutes after speed was attained at 52,640 r.p.m. The near identity of these distributions suggests that photos taken after forty-eight minutes can be used to represent the distribution at infinite time. This not only shortens the experimental runs, but it also greatly reduces the attendant calculations. Because we are interested only in gross differences in distribution between the linear and branched dextrans, this short-

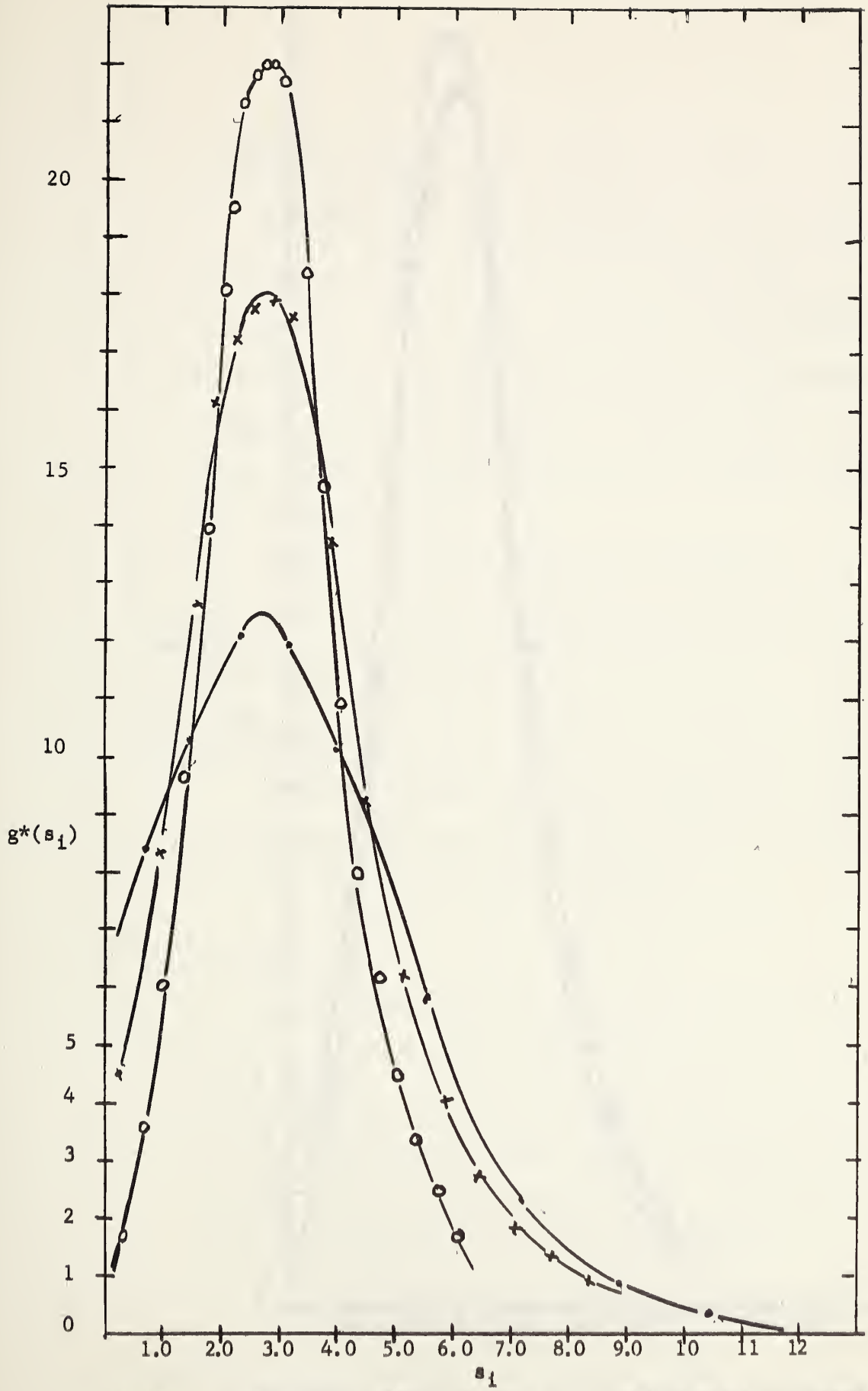


Figure 8. Distribution function $g^*(s_i)$ for sedimentation constants s_i of branched dextran in an aqueous solution of 0.6%. --o-- 48 min. --x-- 24 min. --•-- 8 min. after speed attained (52,640 rpm).

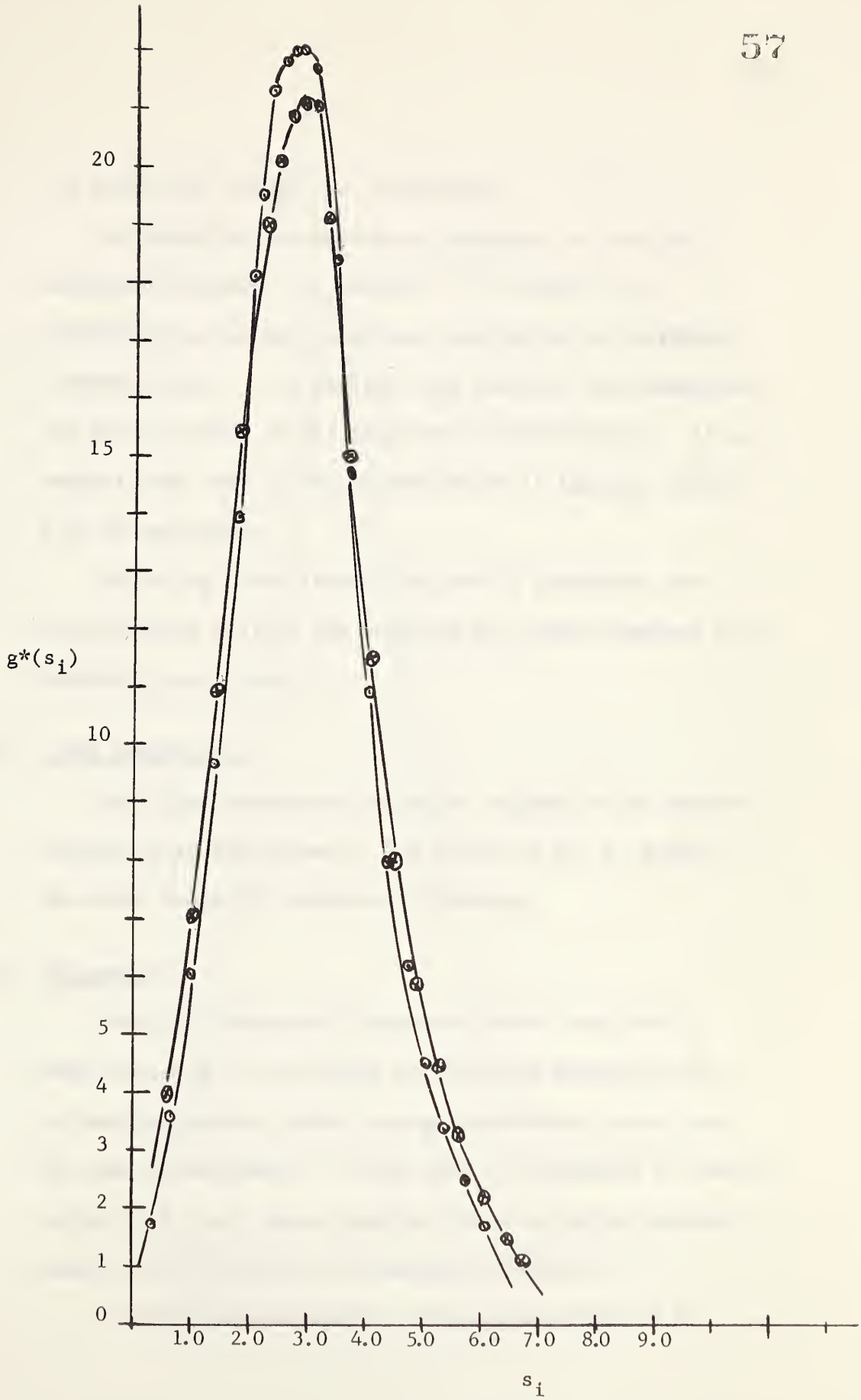


Figure 9. $g^*(s_i)$ vs s_i: 0.6% aqueous solution of branched dextran.
--⊕-- 48 min. --⊗-- 40 min. after speed attained (52,640 rpm).

cut should not affect our conclusions.

Although the concentration dependence of the sedimentation constant of dextran is slight, (3), sedimentation velocity runs were carried out at different concentrations. In Figure 10 are plotted, for comparison, the g(s) function at the different concentrations. It is evident that even if the concentration is ignored, errors will be negligible.

Employing these simplifications of procedure, the distributions g(s) of the branched and linear dextrans were obtained (see Figure 11).

(i) Light Scattering

The light scattering molecular weights of the dextran sulfates were determined in 0.1 M NaCl by Dr. W. Bushuk of the Grain Research Laboratory, Winnipeg.

(j) Viscometry

Modified Ubbelohde Viscometers U-14-2 and U-14-3, made according to the design of Craig and Henderson (96) to have negligible kinetic energy corrections, were used for most measurements. This type of viscometer is ideally suited to a study where numerous dilute solution viscosity measurements are made at successive dilutions.

A multi-bulb viscometer, designed and employed by

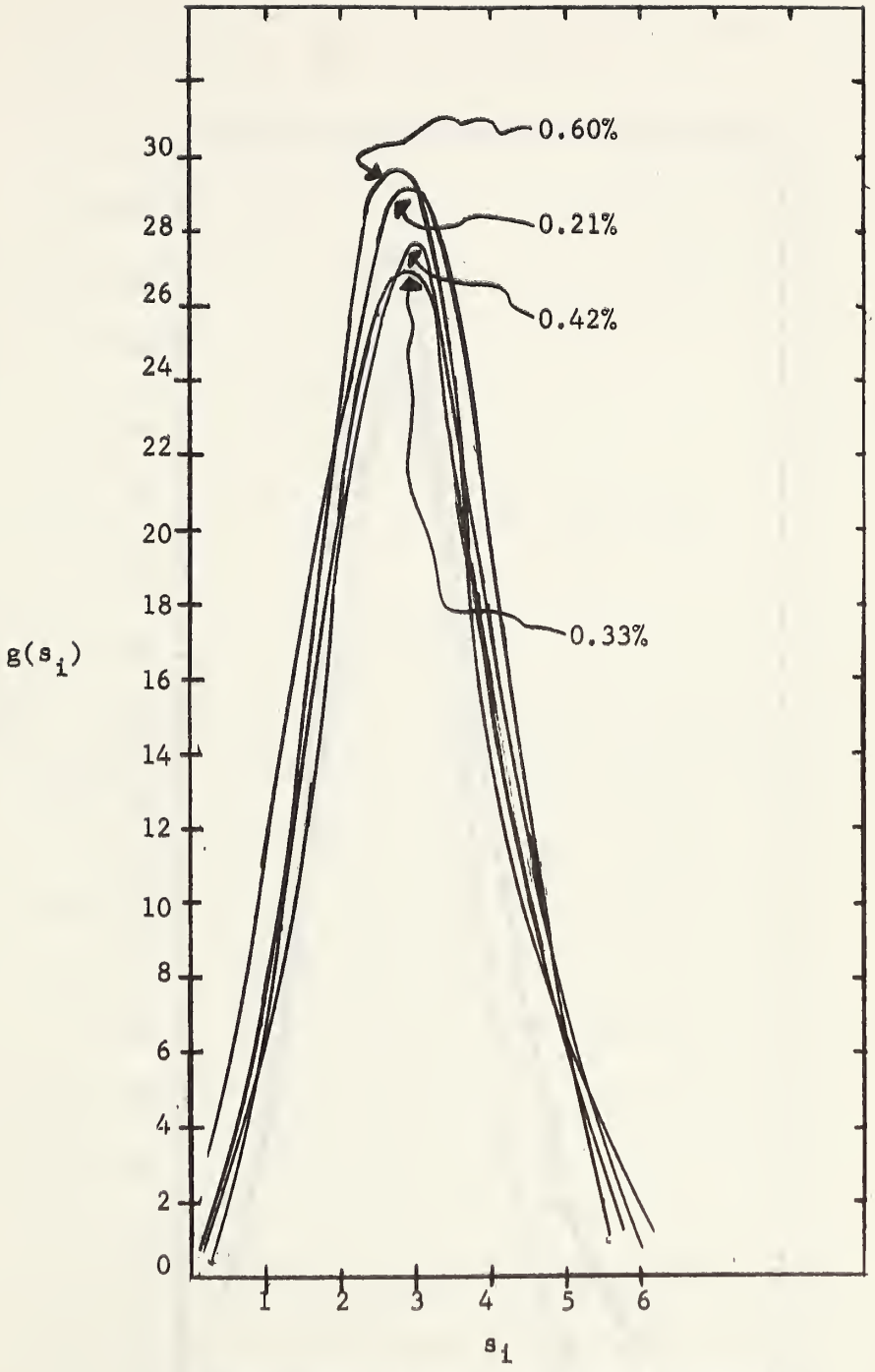


Figure 10. $g(s_i)$ vs s_i: solutions of branched dextran in water of concentrations indicated.

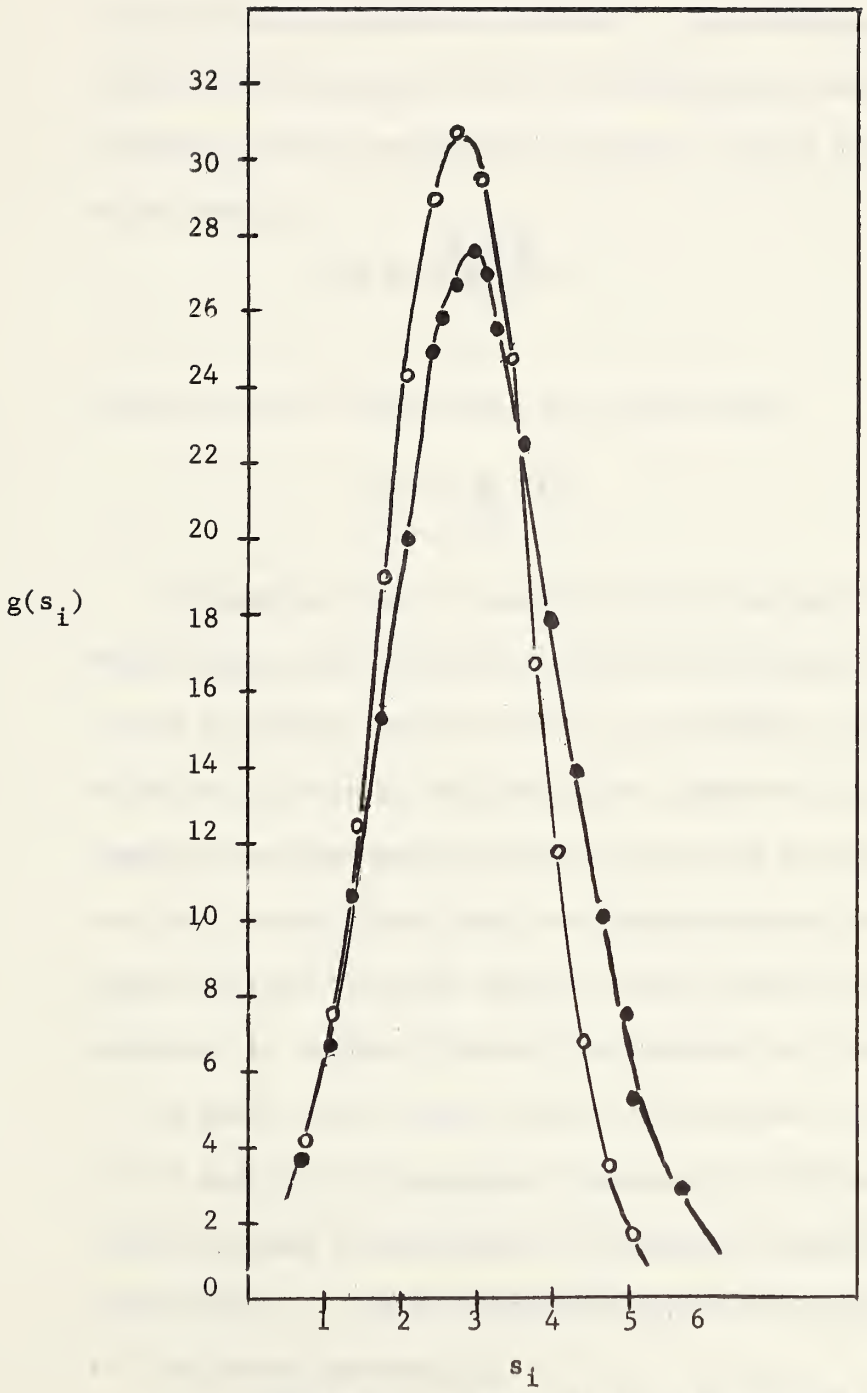


Figure 11. $g(s_i)$ vs s_i: solutions of linear (o) and branched (●) dextrans of concentration 0.42%.

Cragg and Van Oene (97) and Van Oene and Cragg (98) for their studies on unsubstituted dextran, was used for the shear dependence studies. The shearing stress was calculated, from the experimental data obtained with the multibulb viscometer, using the relationship

$$\tau = \frac{hgdR}{2L} \qquad \ldots 16$$

and the rate of shear using the relationship

$$\tau = \eta_n D \qquad \ldots 17$$

Viscometers were cleaned in the following way. Warm chromic acid cleaning solution was allowed to stand in the viscometer several hours, or overnight, after which the viscometer was emptied as completely as possible and thoroughly rinsed, first with hot water and then several times more with demineralized water, then acetone which had been filtered through a very fine (VF) sintered filter and dried by drawing filtered air through it by suction.

A small white plastic plate was attached to the U-14-2 and U-14-3 viscometers behind the bulbs and the fiducial lines in order that the meniscus might be seen more sharply. (This attachement could not be used for the shear viscometer.)

The constant temperature bath consisted of a large cylindrical pyrex vessel, fitted with an American Instrument Electronic Relay (4-530) and a "metastatic" mercury thermoregulator. The bath liquid was water. An electronically controlled GI 21 laboratory mixer with a 3" propeller on a 14" shaft agitated the bath water satisfactorily. Temperatures were measured with a Beckman thermometer. Temperature was controlled to better than $\pm 0.02^{\circ}C$.

A rigid viscometer support, fabricated from a 1" x 1" brass bar was fitted across the bath and attached to its walls by means of specially made clamps. A clamp, semi-permanently attached to the viscometer, having a 3/8" brass rod which fitted a slightly enlarged 3/8" hole in the brass bar, allowed the viscometer to be reproducibly mounted for successive viscosity runs.

Coarse, sintered glass crucibles were used for filtering all solutions before the viscosities were measured. The pipets used were of Exax blue-line quality.

Lemania and Heuer stopwatches, which allowed time determinations to be made to $\pm$ 0.03 seconds, were employed.

The polymer solutions were made up directly to a

concentration of approximately 1% by adding the required volume of solvent (water, N/1000 KCl or N/2000 KCl) to a weighed amount of polymer. After the solution was filtered through a dry coarse sintered glass crucible, its concentration was immediately determined. (This procedure was necessitated by the extreme hydroscopic nature of the polymers.) Duplicate one-milliliter aliquots of the solution were transferred to platinum boats of known constant weight and evaporated to dryness at 80°C in a vacuum oven at atmospheric pressure. When dry, the boats (and films of polymer) were subjected to a partial vacuum of 28 lbs. at 90°C overnight, after which they were allowed to cool in a desiccator containing phosphorous pentoxide as desiccant. The concentration of the solution was calculated from the weight of the polymer residue.

Ten milliliters of the filtered solution was pipetted into the dry Ubbelohde viscometer, and the capillary was rinsed three times before the viscosity flow time was recorded. Dilution was effected by pipetting an appropriate volume of solvent (H_2O, N/1000 KCl or N/2000 KCl) into the viscometer. After the addition, the viscometer was withdrawn from the bath and shaken, put back into the bath, and the capillary

again rinsed three times with the more dilute polymer solution before the viscosity flow times for this solution were measured. This dilution procedure was continued until the desired number of viscosity determinations had been made.

The usual procedure, of taking flow time ratios as equal to viscosity ratios -- rather than converting the flow times to viscosity coefficients and then taking the ratio -- was used throughout this study. This procedure is justified because kinetic energy effects are known to be negligible in the viscometer and because the densities of the dilute polymer solutions differ very little from the density of the solvent.

Flow time measurements were made, at $25^{\circ}C \pm 0.02^{\circ}C$, with the following systems:

(i) Linear dextran in water
Branched dextran in water

(ii) Various dextran sulfates in water

(iii) Various dextran sulfates in N/1000 KCl and N/2000 KCl

(iv) Dextran sulfates in 0.02364 N KCl

(v) Various carboxymethyl dextrans in N/1000 KCl and N/2000 KCl

Viscosity data is to be found in Tables II - XVIII.

D. RESULTS AND DISCUSSION

I. Degradation in the Preparation of the Polyelectrolytes

For a meaningful comparison of the viscosity behaviour of branched and linear polyelectrolytes, it is obviously necessary that the molecule of the branched polyelectrolyte be significantly more branched than that of the linear polyelectrolyte. In this investigation, the dextran polyelectrolytes were prepared directly from dextran samples, one of which was known to be essentially linear, the other extensively branched. Conversion conditions were such as to minimize degradation and branch hydrolysis; the sulfation procedure was deliberately modified to achieve this end. Nevertheless, it was imperative to demonstrate that there remained an appreciable difference in the degree of branching in the "linear" and "branched" dextran polyelectrolytes.

(a) Potassium Carboxymethyl Dextran

With the unsubstituted linear and branched dextrans, a difference in the degree of branching is clearly evidenced in their infrared spectra in the region of 790 $cm.^{-1}$ (see Figure 12). Introduction of carboxymethyl groups does not affect the IR spectra in the region, and a comparison of the spectra for the branched

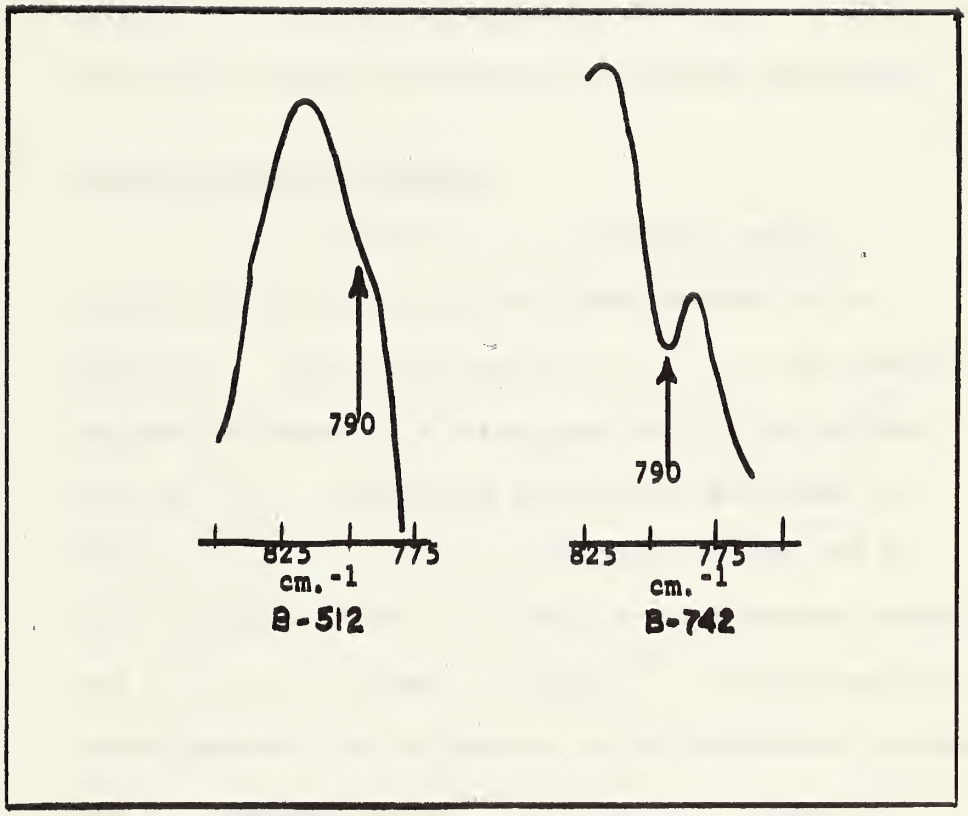


Figure 12. Infrared spectra of linear (B-512) and branched (B-742) dextrans.

and linear carboxymethyl dextrans (see Figure 7) reveals no difference in branching in the branched polyelectrolyte and the parent branched polymer. However, it does reveal an appreciable difference in branching between linear and branched carboxymethyl dextrans -- a difference comparable to that observed for the parent dextrans.

(b) Potassium Dextran Sulfate

A direct comparison of the infrared spectra of sulfated and unsubstituted branched dextran is not possible. The branch peak (790 $cm.^{-1}$) of the dextran sulfate is masked by a strong peak due to the sulfate (810 $cm.^{-1}$). Difference spectra, as described in Section C III g, for the two dextrans and the two sulfate derivatives (of 9% S) used in the viscosity comparison (D IV c), are shown in Figure 6. For the unsubstituted dextrans, the difference in the absorbance between the 1,3 linkage (790 $cm.^{-1}$) and the 1,6 linkage (766 $cm.^{-1}$) was 0.37 absorbance units (see G10), while for the dextran sulfates the difference was 0.25 absorbance units. Taking into consideration the difficulty of obtaining identical pellets and the fact that the sulfate pellets had a slightly smaller concentration of dextran, the results must be considered in reasonably good agreement. At best, they indicate little branch hydrolysis, at

worst they prove that the branched polyelectrolyte is still considerably more branched than the linear one.

The intrinsic viscosities of the linear and branched dextran sulfate of the same degree of substitution (same % sulfur), used in the viscosity comparison of section D IV c, were determined in 0.02364N potassium chloride. The reduced viscosity curves are reproduced in Figure 13, (together with those of the unsubstituted dextran in water). It is evident that in this relatively concentrated salt solution, a large part of the polyelectrolyte effect has been "swamped out". (Compare the curves obtained with these same samples with N/1000 KCl as solvent (Figures 28 and 29)). The residual polyelectrolyte effect should affect the linear and branched macromolecules to approximately the same extent and merely amplify the initial difference between the parent linear and branched dextrans. If any degradation had occurred during sulfation, it is highly unlikely that it would occur to the same extent in both the linear and branched dextran systems. Therefore, if appreciable degradation had occurred, one would expect the ratio of the intrinsic viscosities (linear to branched) to be significantly different for the dextran sulfates than for the parent dextrans. The ratio of the intrinsic viscosities

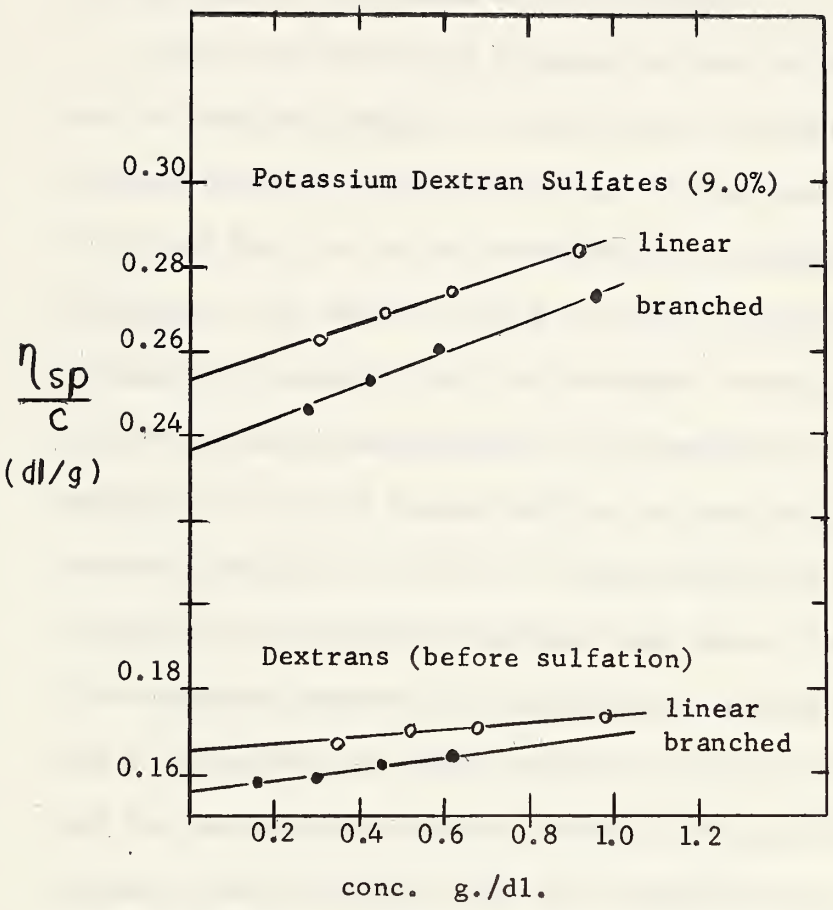


Figure 13. Reduced viscosity vs concentration: dextrans in water, and dextran sulfates in 0.02364N KCl.

(linear to branched) is 1.04 for the dextrans and 1.07 for the dextran sulfates. The difference is negligible. Hence it may be concluded that little or no degradation took place during sulfation.

Since the linear and branched sulfate derivatives are of identical degree of substitution and their reduced viscosities were determined in the same solvent (0.02634N KCl), it is not unreasonable to compare their k′ values (see Section B II a) to obtain further viscometric evidence that the "branched" dextran sulfate is more branched than the "linear" one. Huggins' k′ for the linear dextran is 0.45; for the branched dextran it is 0.57 -- the larger value being evidence for branching consistent with other evidence from infrared spectroscopy and periodate analysis. The k′ value for the linear dextran sulfate is 0.54 and for the branched dextran sulfate 0.68; again the larger value is evidence that the "branched" polyelectrolyte is indeed more branched than the "linear" polyelectrolyte.

After sulfation and purification, the molecular weights of the two dextran sulfates were determined by light scattering in aqueous 0.1N NaCl. The values obtained were 104,000 for the branched sample and

69,000 for the linear sample. Since 9% sulfur is equivalent to 0.68 ionic groups ($-SO_2OK$) per anhydroglucose unit (A.G.U.), this means that each mole of anhydroglucose unit gains 80.9 grams after sulfation and weighs 243.04 grams. Therefore, if one takes a mole of unsubstituted branched polymer which has a $\bar{M}_w$ of 61,000 (the molecular weight of our sample) one has taken 376 moles of A.G.U. which, after sulfation, should weigh 91,500. Similar calculations for the linear sample predict a molecular weight of 58,500 for an unsubstituted linear dextran molecular weight of 39,000 (the molecular weight of our sample).

The experimental values, though higher than the calculated values, do not differ from them by more than experimental error of light scattering measurements. The discrepancy certainly cannot be due to degradation for degradation would result in lower rather than higher values in both samples. Moreover, with random scission of 1,6 linkages in the backbone chain, even scission of 1% of the linkages would reduce the average degree of polymerization (DP) -- and therefore the average molecular weight -- to a value less than half its original value. (The molecular weight of 39,000 of our linear dextran corresponds to a DP of about 240 and

scission of 1% of the 1,6 linkages would therefore correspond, on the average, to more than 2 breaks in the backbone chain.) The light scattering evidence therefore supports the conclusion that little or no degradation has occurred.

Sedimentation constants of 1% solutions of the linear dextran in water and of the linear dextran sulfate (9% S) in 0.1N KCl (corrected, for the temperature and viscosity of the solution, to standard conditions) were 2.09 and 2.85. The sedimentation constants for the branched dextran and branched dextran sulfate (9% S) were 2.56 and 3.32. These results indicate the expected increase in mass after sulfation. Any degradation -- chain scission or branch hydrolysis -- would have caused the sedimentation constants of the sulfated material to be smaller than the sedimentation constants of the parent polymers.

Convincing evidence that neither backbone degradation nor branch hydrolysis occurred during sulfation is obtained from proton magnetic resonance studies on linear and branched dextrans and dextran sulfates (see Section D II). From these it may be concluded that the extent of degradation is not more than -- and is probably much less than -- 10% whether in linear dextran (breaking of

1,6 linkages in the chain) or in branched dextran (breaking of 1,6 linkages plus breaking of the non-1,6 linkages at branch points).

From the viscosity, light scattering, and ultracentrifuge evidence, as also from the PMR evidence, it is clear that essentially no degradation of the backbone chain, either of the linear dextran or the branched dextran occurred during sulfation. It is safe to conclude, too, that no degradation would occur in the milder process of carboxymethylation.

Although for the purposes of this study, it would have been sufficient to demonstrate a difference in the degree of branching in the "linear" and "branched" polyelectrolytes, we can go further. From the IR evidence obtained with the dextran sulfates and the carboxymethyl dextrans and the PMR evidence for the dextran sulfates, it is clear that very little, if any, branch hydrolysis occurred during the preparation of the polyelectrolytes.

All the evidence indicates that not only is there a significant difference in the branching, but also that very little, if any, degradation or branch hydrolysis had occurred during the preparation of the polyelectrolytes and, hence, that the difference in branching of the polyelectrolytes is essentially the (known) difference in

branching in the parent dextrans.

II. Proton Magnetic Resonance (PMR) and Branching

(a) Linear and Branched Dextran

Structures of linear and branched dextran established on accumulated chemical evidence (see Section B III) are shown diagrammatically in Figures 14 and 15. (Because there is slight predominance of 1,3 branching in the branched sample (see Section C II) this linkage was chosen to represent the branching linkages.) Examination of the diagram suggests that, because of differences in the environs of the protons of the two compounds, proton magnetic resonance (PMR) spectra might exibit significant differences which could be related to branching. If this were so, then an extension of the analysis of the dextran spectra to the dextran sulfate spectra might throw light on the fate of the branches during sulfation.

Isomaltotriose was selected as a model compound to aid in peak assignments because of its close structural similarity to linear dextran (compare Figures 14 and 16). Its PMR spectrum is reproduced Figure 17. Peak 4 at -4.80 ppm is due to the

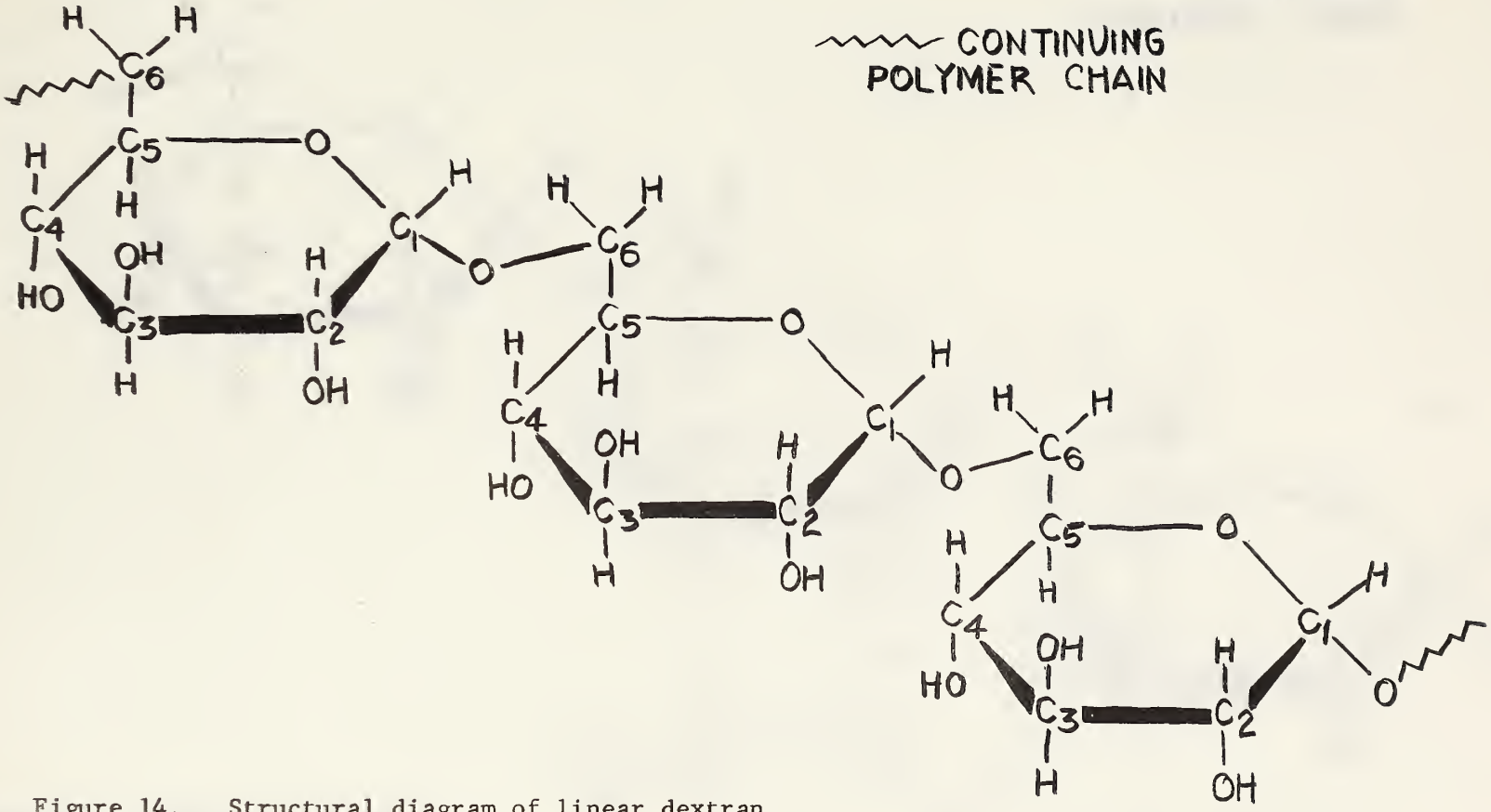


Figure 14. Structural diagram of linear dextran.

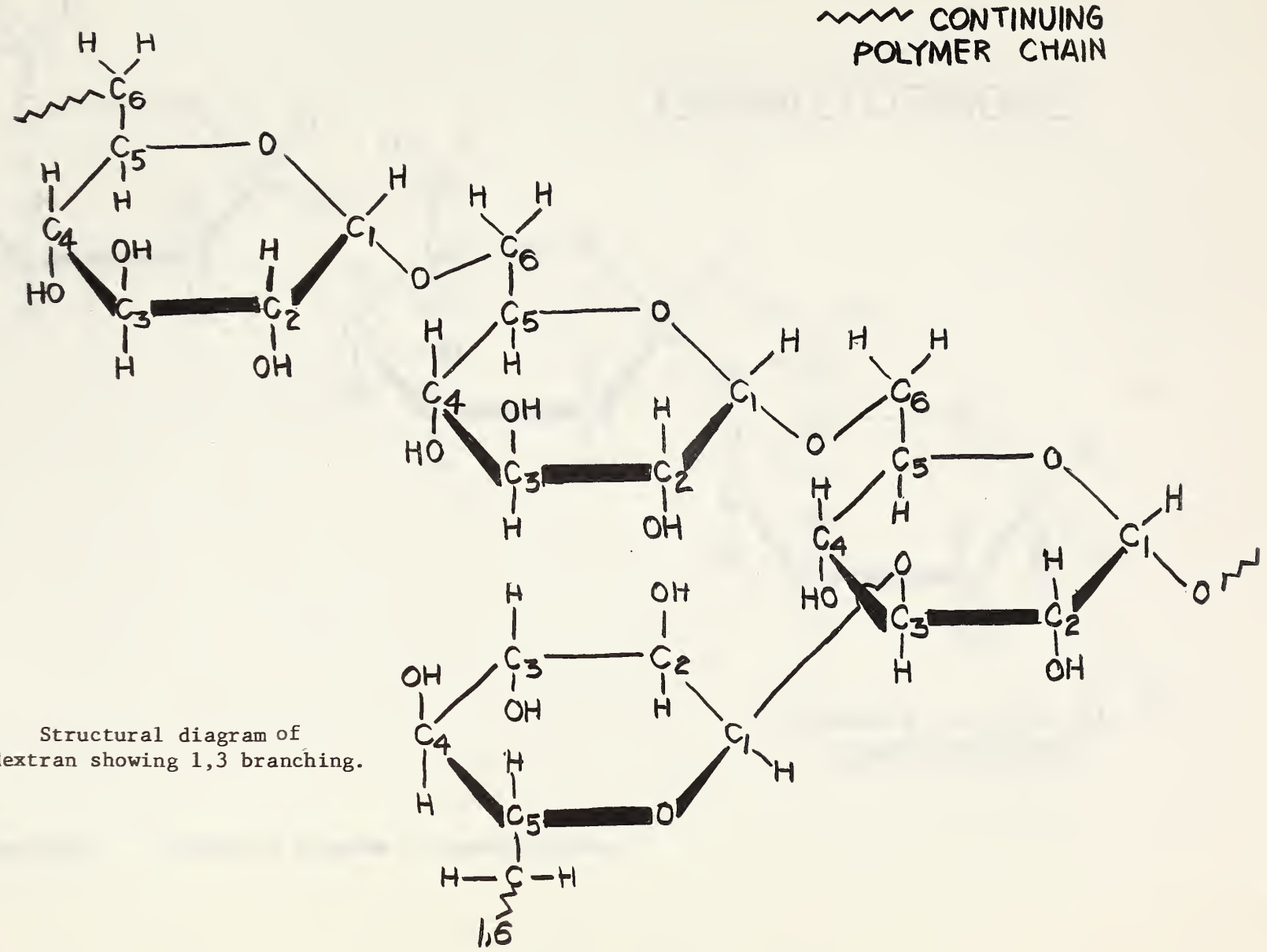


Figure 15. Structural diagram of branched dextran showing 1,3 branching.

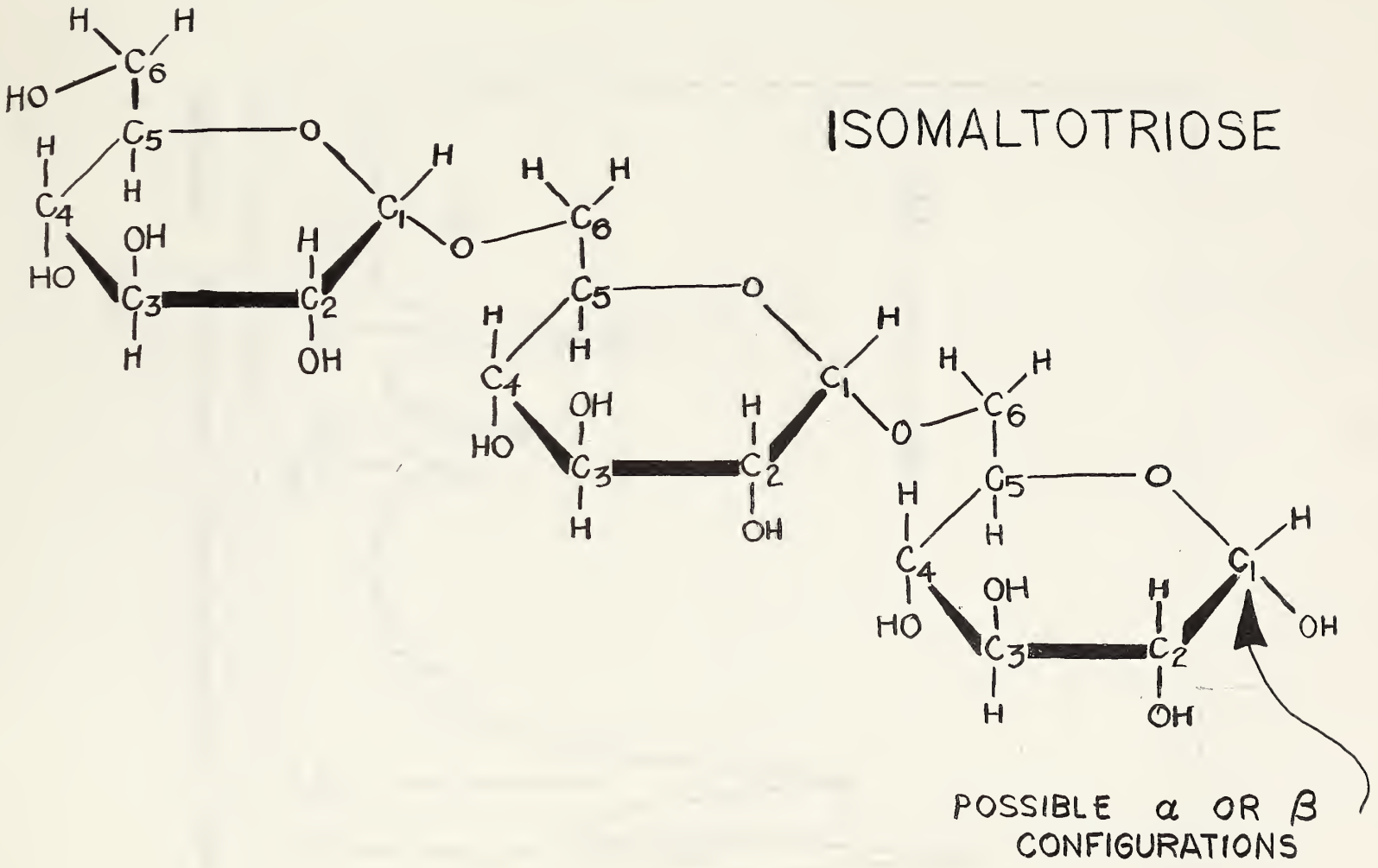


Figure 16. Structural diagram -- isomaltotriose.

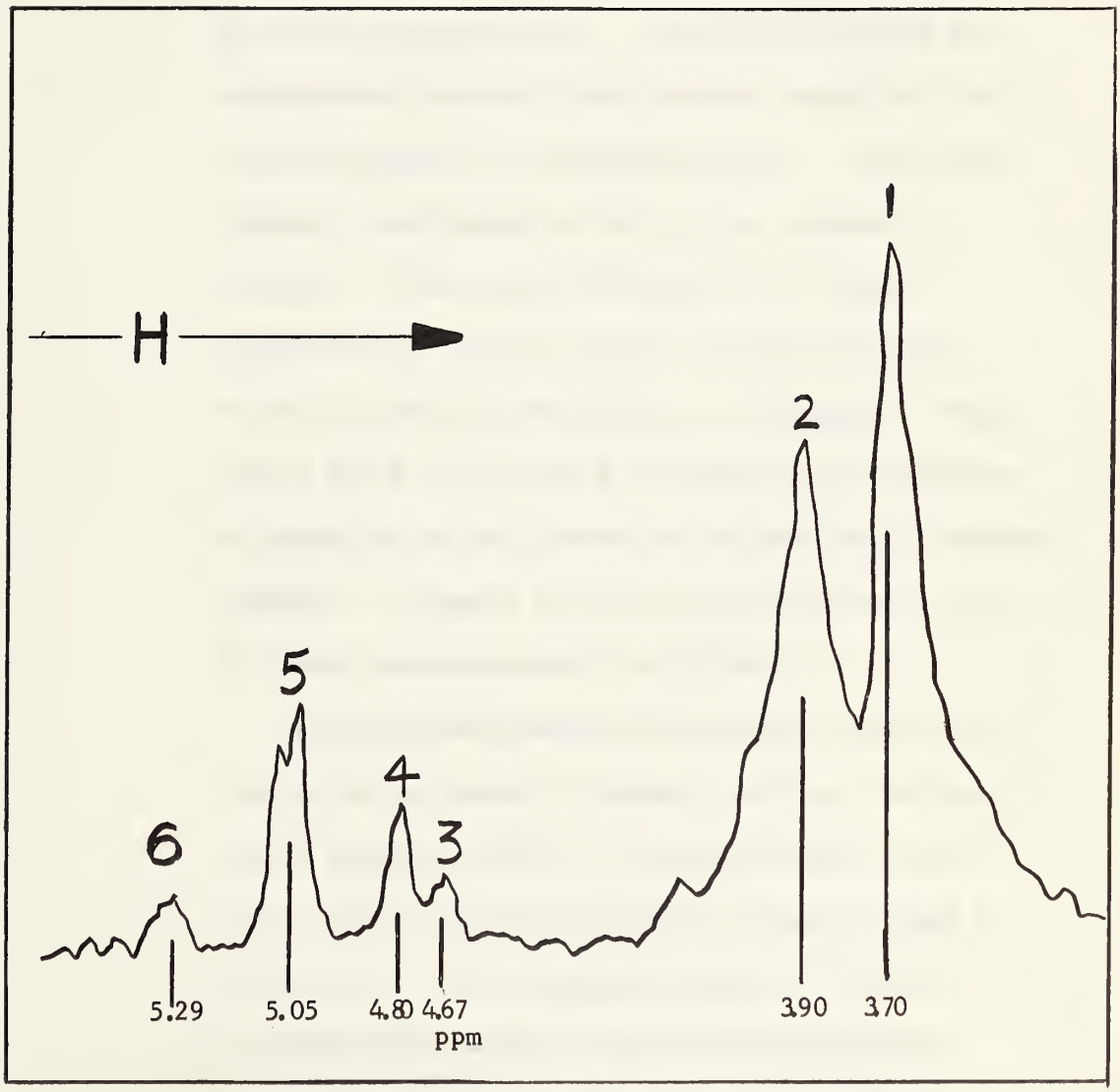


Figure 17. Proton magnetic resonance spectrum of isomaltotriose.

residual protons in the deuterium oxide which was used for the solvent. The relatively intense signals 1 and 2 (-3.70 and -3.90 ppm) may be attributed to the C_5 and C_6 protons and the C_2, C_3 and C_4 protons respectively. (The protons with the environments having higher electron densities would give the signal at the higher field.) The other signals, then, would be due to the anomeric C_1 protons. The size of signal 5 (-5.05 ppm) suggests that it must be due to the two protons on the C_1 carbons involved in 1,6 linkages. Signals 3 and 6 (-4.67 and 5.29 ppm) are then assigned as being due to the proton on the hemi-acetal anomeric carbons. Signals 3 and 6 can be assigned to the β and α configurations (99) (100).

If these assignments are correct, the ratio of the sum of the areas of peaks 1 and 2 to the area of peak 5 should be 18/2 or 9, and the ratio of the area of peak 5 to the sum of the areas of 3 and 6 should be 2. The measured ratios, 9.0 and 2.2 are in satisfactory accord with these predications. (Areas of the respective peaks are proportional to the number of protons responsible for the particular signal (100) (101).

The spectrum of the linear dextran NRRL B-512 is reproduced in Figure 18. In this high-polymeric polyglucose, the contribution to the PMR spectrum of the anomeric proton on the reducing end would be vanishingly small. It would be expected, therefore, that peaks 3 and 6 would not appear. In fact, they do not. The three signals that are observed correspond to the signals 1, 2, and 5 of the isomaltotriose spectrum, and are so labelled. Making an assignment analogous to that for isomaltotriose, one would predict that the ratio of the sum of the areas of peaks 1 and 2 to the area of peak 5 would be 6/1 or 6. The value obtained from area measurements was 5.9.

The PMR spectrum for branched dextran NRRL B-742, reproduced in Figure 19, contains peaks corresponding to those in Figure 18, but, in addition, has a peak, 7, at -5.40 ppm, whose counterpart does not occur in the spectrum of isomaltotriose or linear dextran. That the peaks other than 7 are indeed counterparts of one another is evident from the data of Table XIX. Differences in structure of the three compounds have affected the chemical shift values very little. This new

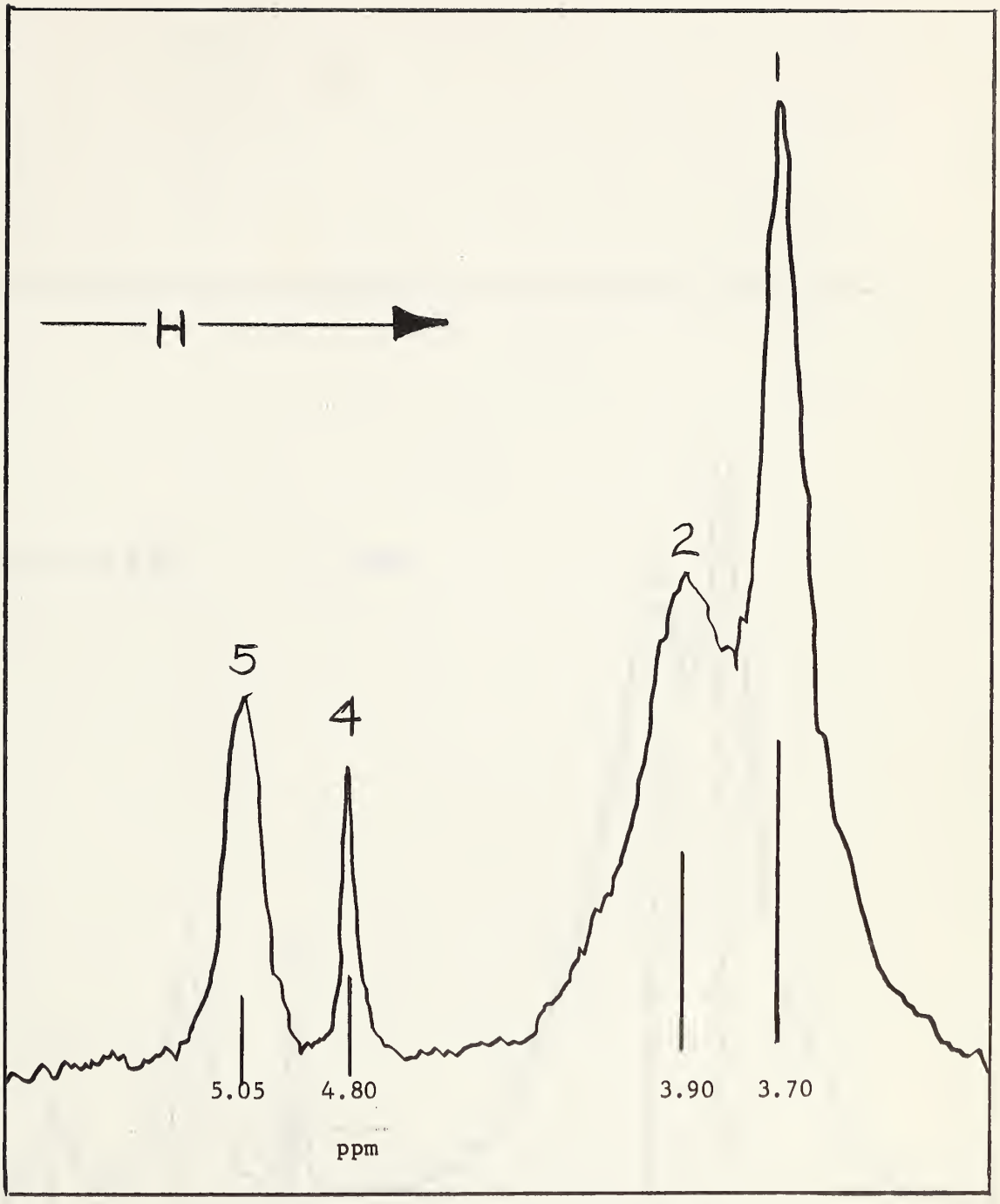


Figure 18. Proton magnetic resonance spectrum of linear dextran.

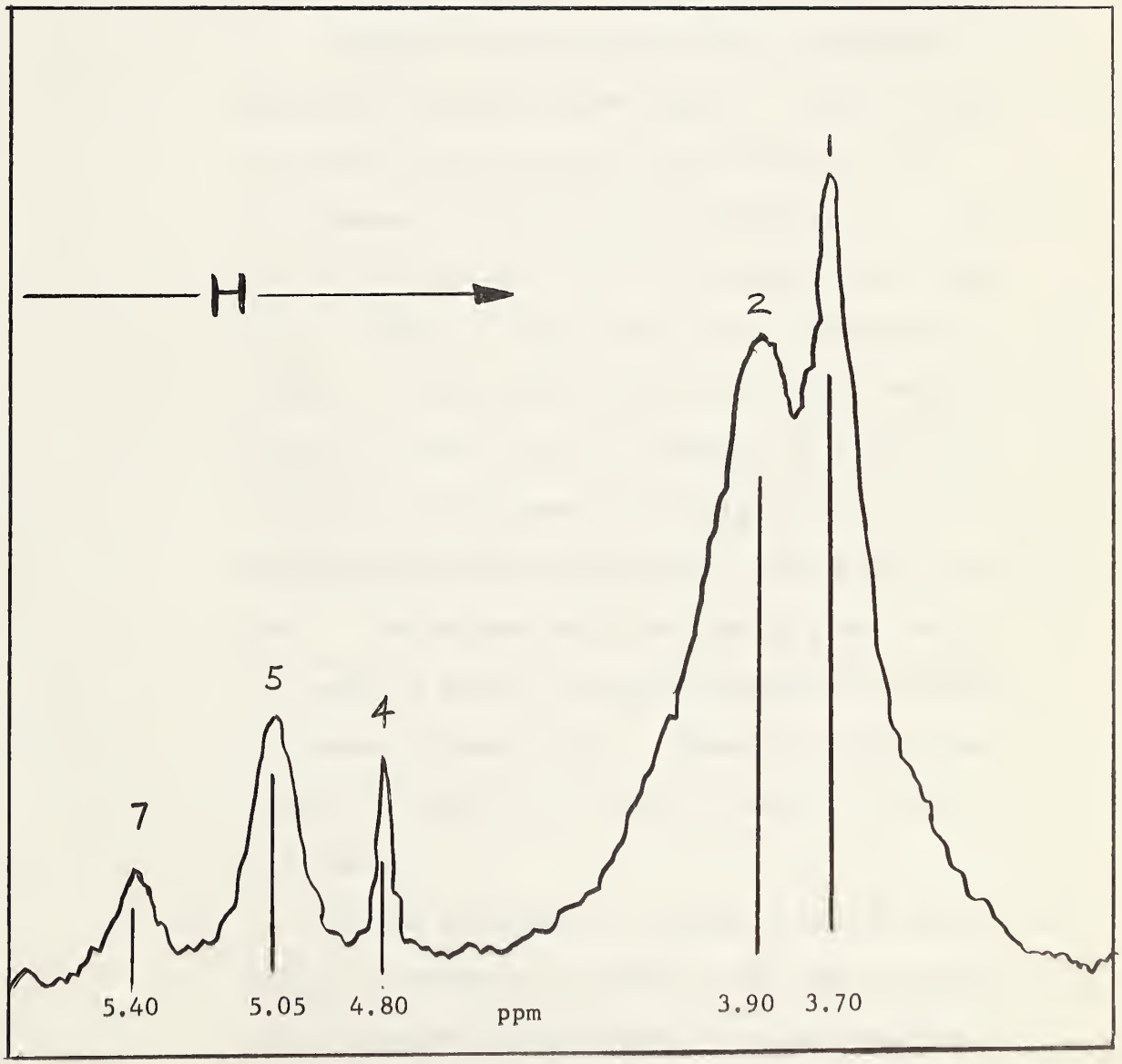


Figure 19. Proton magnetic resonance spectrum of branched dextran.

peak can only be due to the one structural feature -- non-1,6 linkages -- known to be present in B-742 dextran (45) (47) but absent from isomaltotriose and present only to a very small extent (less than 5%) in B-512 dextran. (In Figure 15 these "non-1,6 linkages" are represented by a 1,3 linkage.) The proton responsible for signal 7 must be either the one on the C_1 or else that on the C_3 of the 1,3 linkage (or the C_4 if the linkage is 1,4). Of the two alternatives, the first seems the more probable. Signal 7 must arise at the expense of the signal at 5 (C_1 proton at the 1,6 linkage), with a small shift from -5.05 to -5.40 ppm; or else it is produced at the expense of the signal 2 (C_2, C_3, C_4 protons) with a much larger shift from -3.90 to -5.40 ppm. For neither the C_1 nor the C_3 proton would one expect a change in proton environment sufficient to produce a large shift. Tentatively, then, we assign the signal at 7 to the C_1 proton at the non-1,6 linkage.

If this assignment is correct it should follow that, if comparisons are made at the same concentrations in grams per milliliter (i.e. with the same number of anhydroglucose units in a given volume

of solution):

(a) the combinedintensities of signals 1 and 2 should be the same for the linear and branched dextrans; and

(b) the intensity of signal 5 for the linear dextran should equal the sum of the intensities of signals 5 and 7 for the branched dextran (anhydroglucose units that are joined by 1,3 or 1,4 linkages in the branched dextran would have been joined by 1,6 linkages had the dextran been linear).

In Figure 20, the sum of the areas of peaks 1 and 2 is plotted against concentration for both dextrans. In accordance with prediction (a) above, the points for the two dextrans fall on the same line. In Figure 21 are plotted, against concentration: A, the area of peak 5 (1,6 linkage) for the linear dextran; B, the area of peak 5 for the branched dextran; and C, the area of peak 7 (the "branching peak") for branched dextran. Also plotted are the sums of the areas of 5 and 7 for branched dextran. These last points fall on the same line as do the points representing peak 5 areas for linear dextran, in accordance with prediction (b) above. Thus, the assignment of peak 7 to the C_1 proton of the non-1,6 linkage is confirmed.

Further confirmation is afforded by a comparison of peak areas in the branched dextran spectrum. From periodate oxidation and methylation studies (47),

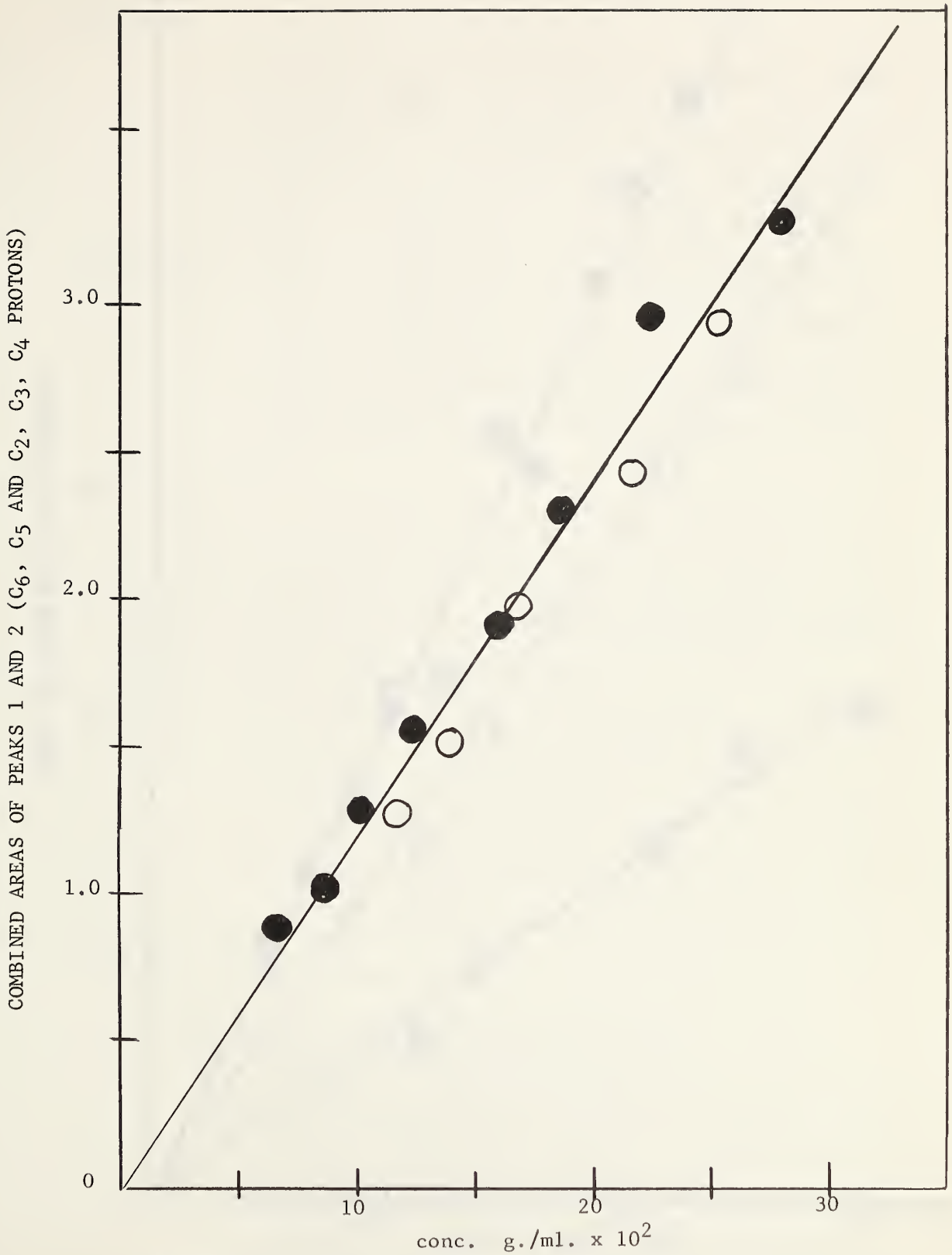


Figure 20. Combined peak areas 1 and 2 for dextran vs concentration.

● linear

○ branched

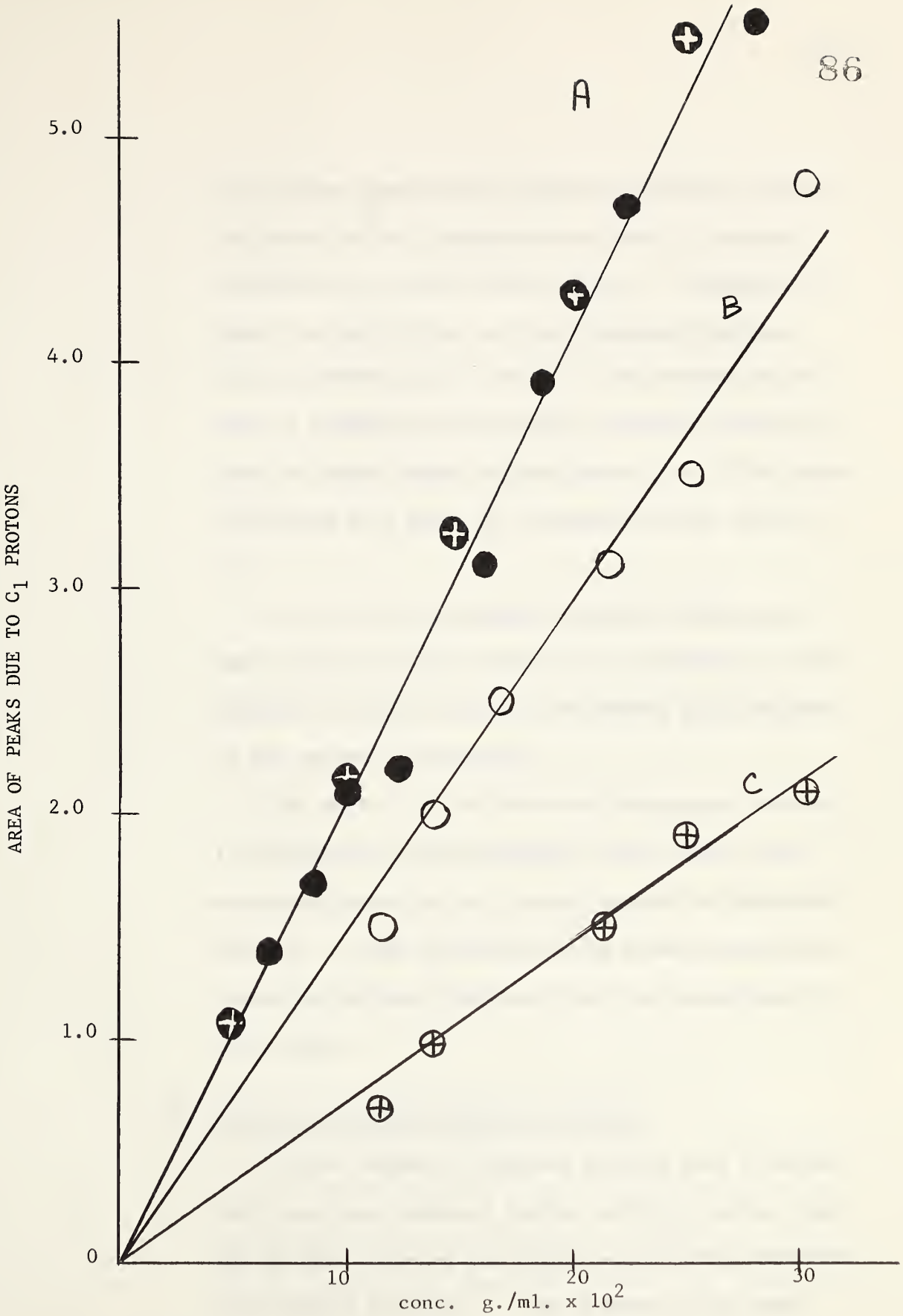


Figure 21. Areas of peaks due to C_1 protons of dextran as a function of concentration.

A. ● 1,6 linkages of linear dextran
B. ○ 1,6 linkages of branched dextran
C. ⊕ non-1,6 linkages of branched dextran
A. ✪ sum of B and C

it is known that in this particular branched dextran the ratio of 1,6 linkages to the non-1,6 linkages is 70/30 or 2.3 (see Section C II). Assignment of peak 5 to the protons on the C_1 carbons involved in 1,6 linkages and of peak 7 to the protons at the non-1,6 linkages would require, therefore, that the ratio of these signal intensities be 2.3 The ratio calculated as a ratio of the measured peak areas is 2.4.

It is to be concluded, then, that PMR spectra may be used not only to detect the branching in this dextran, but also to make a reasonably good estimate of the extent of branching.

The method as used does not distinguish between 1,3-like and 1,4-like linkages, all of which occur at branch points in one form or another of branched dextran. That branching can be detected quantitatively is the more important fact for the purpose of this study.

(b) Linear and Branched Dextran Sulfate

Proton magnetic resonance spectra were obtained for linear and branched dextran sulfates in the same way as were those of the linear and branched dextrans (see Section C III f). The spectra of the deri-

vatives are reproduced in Figures 22 and 23. The peaks are numbered as previously (with one exception, peak 8, which will be discussed later). Peak 4 at -4.7 ppm is due to the proton of deuterium hydroxide. Peaks 1 and 2 in the dextran sulfate spectra occur at the same positions as the corresponding peaks in the dextran spectra (see Table XX they may therefore be attributed to the C_5 and C_6 protons and the C_2, C_3 and C_4 protons respectively (see Figure 14). Similarly peak 5 occurs at the same position in the dextran sulfate spectra as in the dextran spectra (see Table XX); in all spectra it is due to anomeric C_1 protons involved in 1,6 linkages.

Comparison of Figure 22 with 18 and 19 reveals that there is a new peak, 8, in the linear dextran sulfate spectrum that is not present in the spectrum of the unsulfated linear dextran, and that this peak occurs -- unfortunately -- at just the same position as does the branching peak (peak 7) in the spectrum of branched dextran, namely at -5.4 ppm. In the spectrum of linear dextran sulfate (see Figure 22) this peak cannot be due to branching. Rather, it must be due in some way to the sulfate groups, the feature

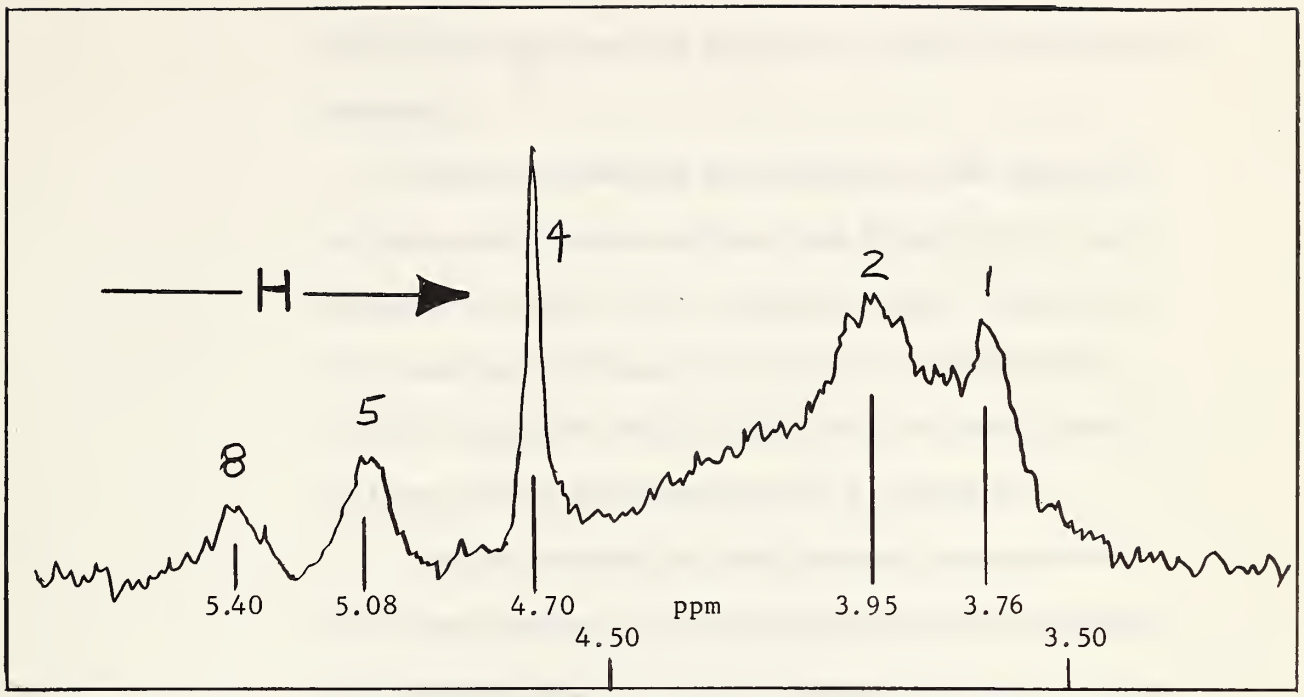


Figure 22. Proton magnetic resonance spectrum of linear dextran sulfate.

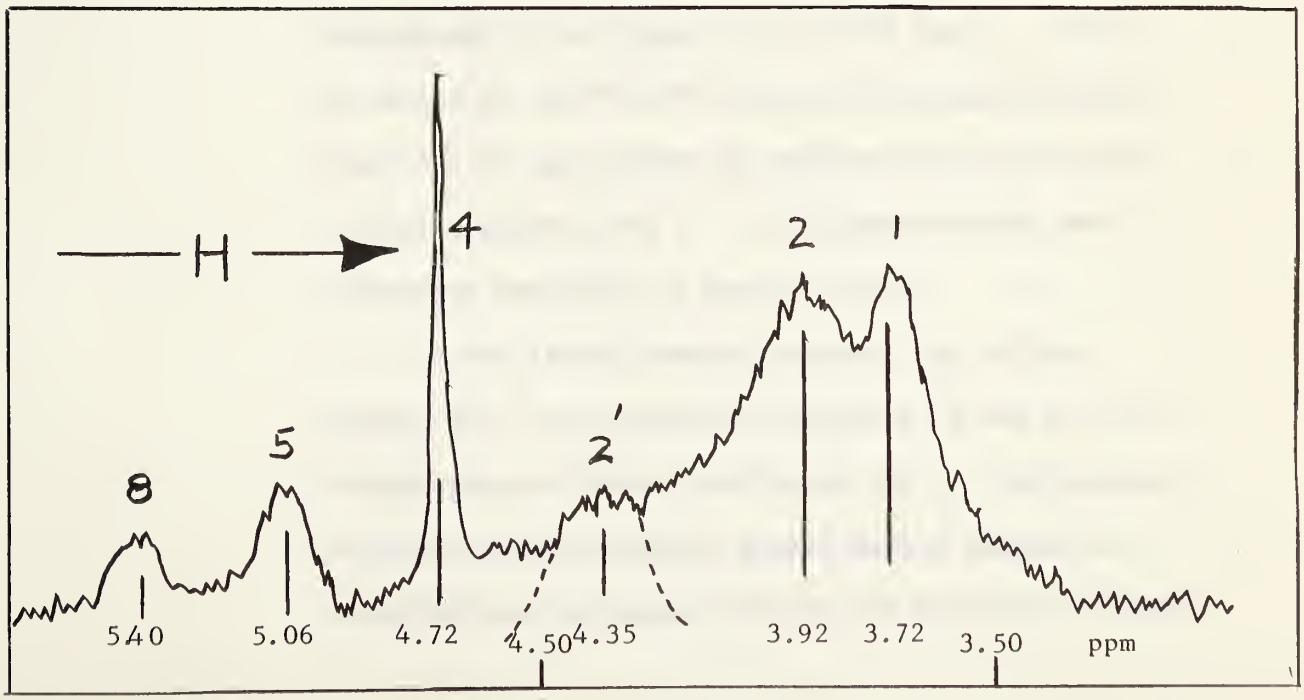


Figure 23. Proton magnetic resonance spectrum of branched dextran sulfate.

that distinguishes the dextran sulfate from the parent dextran.

The corresponding peak occurs in the spectrum of branched dextran sulfate (see Figure 22) -- and again it occurs at -5.4 ppm where peak 7 occurs in the spectrum of branched dextran (see Figure 19). In this spectrum, then, peak 8 must be due to both sulfate and to branching (non-1,6 linkages).

Careful comparison also reveals the existence of a new feature in both the dextran sulfate spectra, a broad shoulder or "tail" between -4.0 and -4.5 ppm. For this, too, the sulfate groups must in some way be responsible. (In the branched dextran sulfate spectrum there is moreover a semblance of a peak, designated 2' in Figure 23, on this tail.) The presence of the "tail" in the sulfate spectra made impractical any attempt to measure the area of the combined peaks 1 and 2. Area measurements were therefore made only on peaks 5 and 8.

In the linear dextran sulfate, the sulfate groups will be attached to carbons 2, 3 and 4 of the anhydroglucose units (see Figure 14). The presence of these electronegative groups should reduce the electron density around the protons attached to these

carbons and hence produce a shift down-field from -3.95 ppm. Since our samples are only partially sulfated, peak 2 would not disappear, but it would be diminished in area with a new signal appearing at its expense.

The sulfate groups should also affect the C_1 protons (those associated with the 1,6 linkages), but here only the sulfate groups on the adjacent carbon C_2, would be expected to cause a shift large enough to produce a separate peak (101a). Again, the effect would be an increased chemical shift from -5.05 ppm; peak 5 would be diminished in favor of a new peak somewhere to its left on the spectrum as drawn.

On this basis, it is reasonable to assign peak 8 to anomeric protons attached to C_1 carbons that are bonded to sulfate-bearing C_2 carbons, and to assign the shoulder between -4 and -4.5 ppm to protons on sulfate-bearing C_2, C_3 and C_4 carbons. Peak 8, then, would be formed at the expense of peak 5. The alternative would <u>not</u> be reasonable, for assignment of peak 8 to protons on the sulfate-bearing C_2, C_3 and C_4 carbons would imply a very large negative shift from -3.9 to -5.4 ppm, and the consequent assignment

of the "shoulder" to the anomeric protons would imply a most unlikely positive shift from -5.0 to -4.5 ppm.

Evidence for or against degradation was obtained from comparison of peak areas in different spectra. In Figure 24 are plotted, against concentration: A, the area of peak 5 for the linear dextran; B, the area of peak 5 for the branched dextran; and C, the area of peak 7 for the branched dextran. Points representing the sum of the areas of peaks 5 and 7 for branched dextran fall, as expected, on line A; for the same number (or number of moles) of anhydroglucose units, the number of 1,6 linkages and non-1,6 linkages, taken together, in branched dextran should equal the number of 1,6 linkages in linear dextran.

In Figure 25 are plotted against concentration for linear dextran sulfate: B, the area of peak 5; C, the area of peak 8; and Σ, the sum of those areas (as taken from the lines B and C). If there is no degradation (scission of 1,6 linkages) the number of 1,6 linkages per given number of moles of the repeating unit will be the same in linear dextran sulfate as in the parent linear dextran, and hence

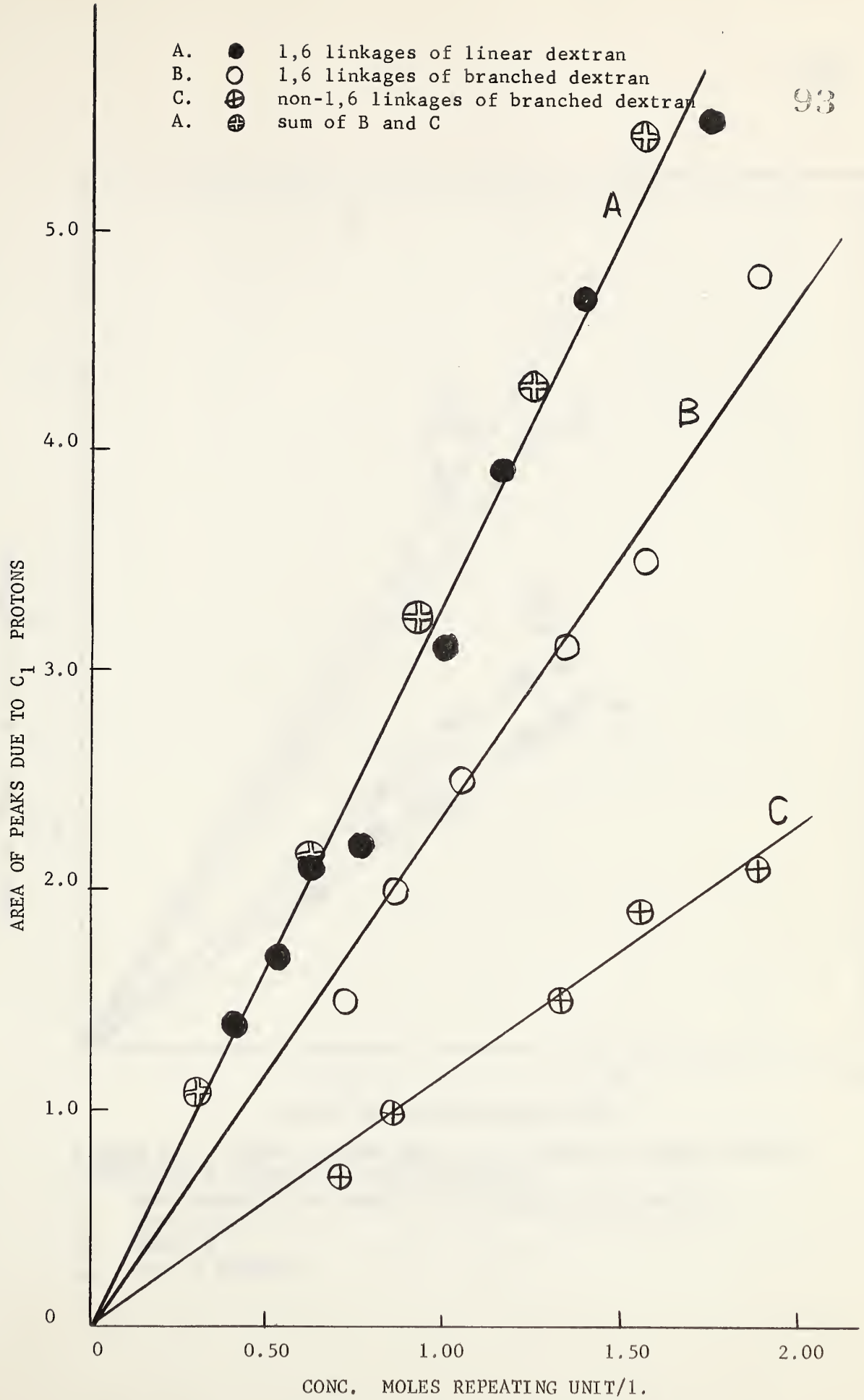


Figure 24. Areas of peaks due to C_1 protons of dextran as a function of concentration.

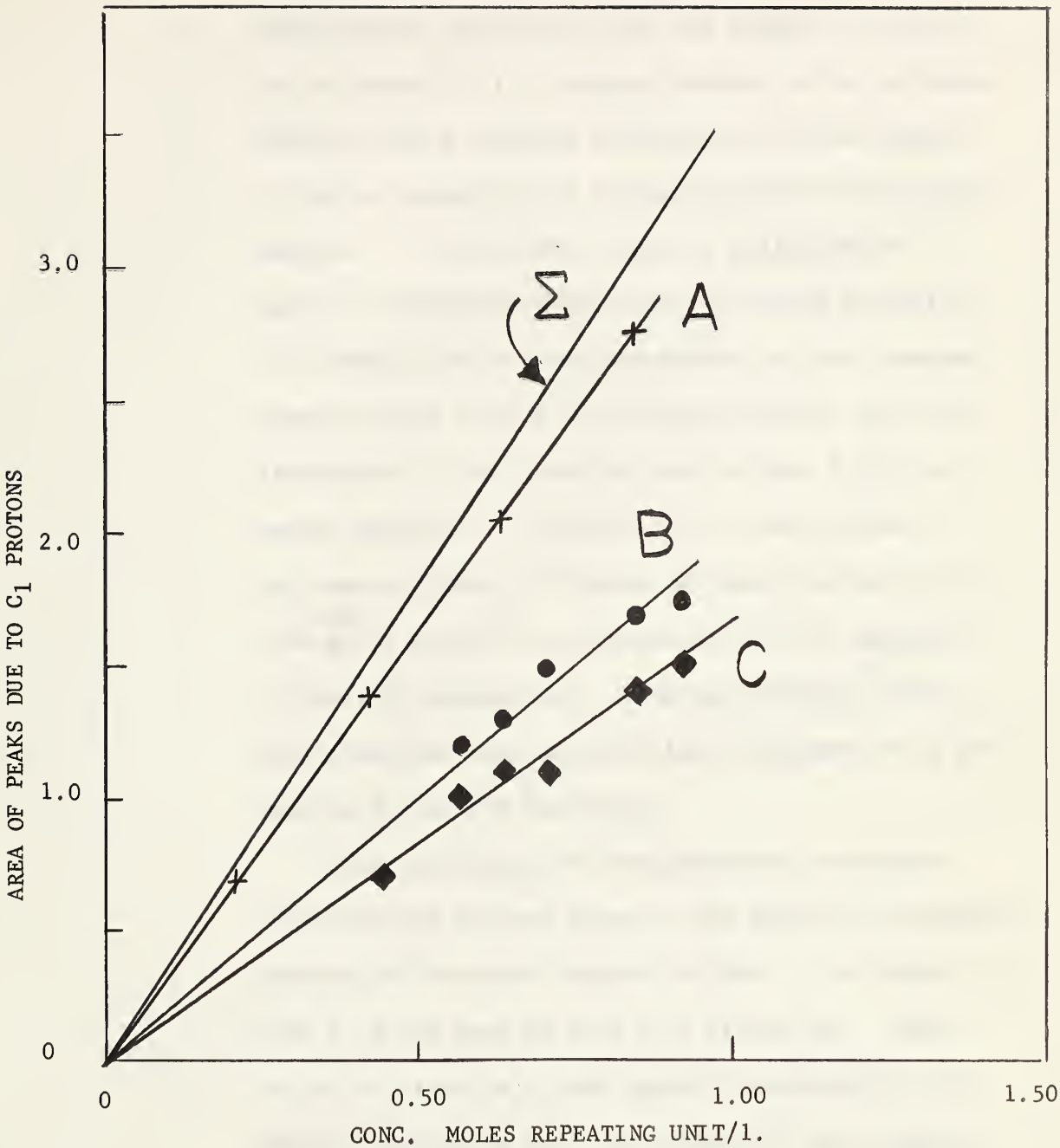


Figure 25. Areas of peaks due to C_1 protons in linear dextran sulfate as a function of concentration.
A. area expected for peak 5 for unsubstituted dextran
B. peak 5
C. peak 8
Σ sum of B and C

the combined intensity of the two signals (5 and 8) due to anomeric (1,6 linkage) protons in the sulfated dextran should equal the intensity of the one signal, 5, due to anomeric (1,6 linkage) protons in the parent dextran. On the other hand, if a significant amount of degradation had occurred during sulfation, 1,6 linkages would have been broken and the combined areas of peak 5 and 8 for sulfated dextran would be significantly less than the area of peak 5 for the parent dextran. In Figure 25 , line A (which is the same as line A of Figure 24) should coincide with line Σ if there is no degradation, or lie above it if there is degradation. We may conclude, then, that there has been no appreciable degradation of the dextran during the sulfation.

This conclusion is strengthened by a similar consideration of peak areas of the spectra of branched dextran and branched dextran sulfate. In Figure 26 line A is the same as line A of Figure 24. Here it may be taken as a plot against concentration (in moles of repeating units per liter) of the combined areas of peaks 5 and 7 of the branched dextran, i.e. of the total intensity of signals due to anomeric protons (1,6 linkages and non-1,6 linkages). Since

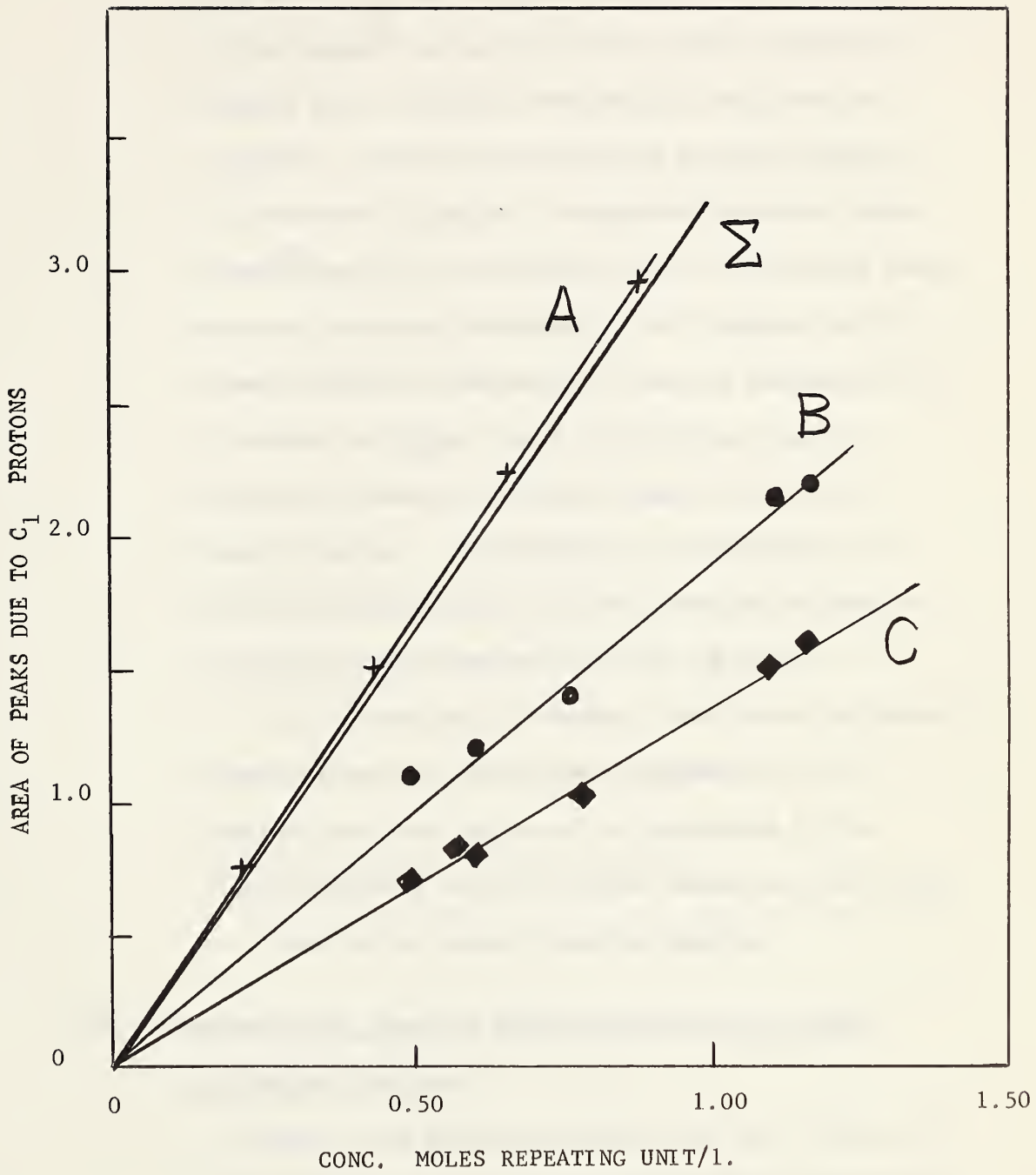


Figure 26. Areas of peaks due to C_1 protons in branched dextran sulfate as a function of concentration.
A. area expected for peak 5 for unsubstituted dextran
B. peak 5
C. peak 8
Σ sum of B and C

line Σ_{is}, for the branched dextran sulfate, a plot of the comparable measure of the total intensity of signals due to anomeric protons (1,6 and non-1,6 linkages), and since the abscissa for both plots is concentration in moles of repeating units per liter, line Σ should coincide with line A if there has been no chain scission (breaking of 1,6 linkages) and no branch hydrolysis (breaking of non-1,6 linkages) and it should lie below line A if there has been an appreciable amount of either branch hydrolysis or chain scission. Considering the imprecision of peak area measurements, the two lines may be said to coincide within experimental error ($\lesssim$ 10%).

It is concluded, therefore, that during sulfation there has been no appreciable degradation of the dextran, and that the extent of branching in the branched dextran sulfate remains essentially the same as it was in the parent branched dextran.

III. Sedimentation Constant Distributions in the Linear and Branched Dextrans

Since it has been established that very little, if any, degradation of any kind occurred during conversion of the dextran, it is safe to assume that the molecular

weight distributions of the sulfated dextrans will be essentially the same as those of their parent compounds. (The same will be true of the carboxymethyl dextrans.)

Sedimentation constant distributions of the linear and branched dextrans were obtained as described under Section C III h. In Figure 11 the distribution curves for the linear and branched samples (completely corrected for diffusion and concentration dependence) are plotted on the same graph. It is evident from this figure that there is no gross difference between these two distributions and, therefore, no gross difference in molecular weight distribution that could be responsible for the large differences in the viscosity behaviour of the linear and branched dextran polyelectrolytes which is described and discussed below.

IV. Viscosity Behaviour of Dextran Sulfates

The reduced viscosities of the samples of potassium dextran sulfate were studied as a function of polyelectrolyte concentration in N/1000 and N/2000 KCl for the reasons mentioned in Section C I.

(a) Effect of Shear Rate

Polymer solution viscosity may be influenced by rate of shear when the dissolved macromolecules

are of very high molecular weight or when they are very stiff (Section B I c). Van Oene and Cragg (98) showed that dilute aqueous solutions of dextran of molecular weight 1×10^7 behaved as Newtonian liquids. As the dextrans used in this thesis were of much smaller molecular weight -- less than 1×10^5 -- their dilute aqueous solutions, too, should be Newtonian. Since, however, conversion of the dextrans to polyelectrolytes might be expected to cause the molecules to become somewhat stiffer, it seemed wise to determine whether or not the viscosity of dilute solutions of these polyelectrolytes exhibited any shear dependence.

Accordingly, the effect of shear rate (D) on the relative viscosity (η_r) for a linear potassium dextran sulfate, of degree of substitution 0.69 sulfate groups per anhydroglucose unit, was determined in 0.1% and 0.5% solution in water and in 0.1% solution in aqueous N/1000 KCl as solvent. The data are plotted in Figure 27 as η_r vs D. Each plot gave a horizontal straight line; the relative viscosity and, therefore, the reduced viscosity, is independent

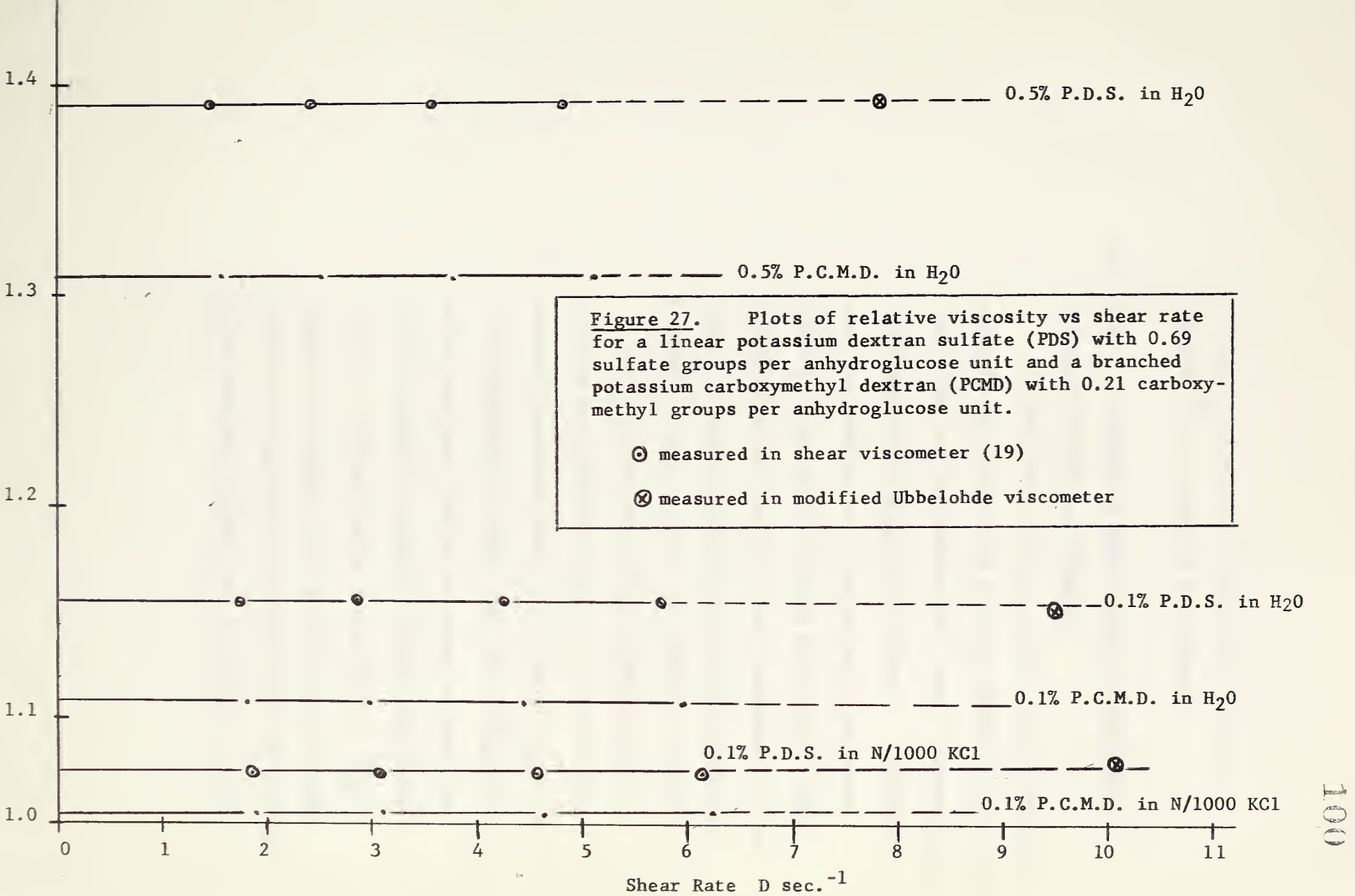


Figure 27. Plots of relative viscosity vs shear rate for a linear potassium dextran sulfate (PDS) with 0.69 sulfate groups per anhydroglucose unit and a branched potassium carboxymethyl dextran (PCMD) with 0.21 carboxymethyl groups per anhydroglucose unit.

⊙ measured in shear viscometer (19)

⊗ measured in modified Ubbelohde viscometer

of shear rate and this factor may safely be ignored in our viscosity measurements.

(b) Effect of Degree of Substitution on the Viscosity Behaviour of Dextran Sulfates

Introduction of sulfate groups into the dextran molecule was expected to increase markedly the reduced viscosities of the macromolecule and to change greatly the shape of the η_{sp}/c vs c curves. In order to obtain an indication of how strongly different degrees of substitution would affect the reduced viscosity-concentration curves, measurements were made in aqueous N/1000 KCl with both linear and branched dextran sulfated to different degrees.

The data for the linear potassium dextran sulfates are plotted in Figure 28 and for the branched potassium dextran sulfates in Figure 29 It is immediately evident that these η_{sp}/c vs c curves are quite similar in shape to those for other solutions of polyelectrolytes (see Section B IV): with decreasing concentration η_{sp}/c climbs to a maximum value and then falls sharply. With increasing degree of substitution, all values of the reduced viscosity are increased in such a

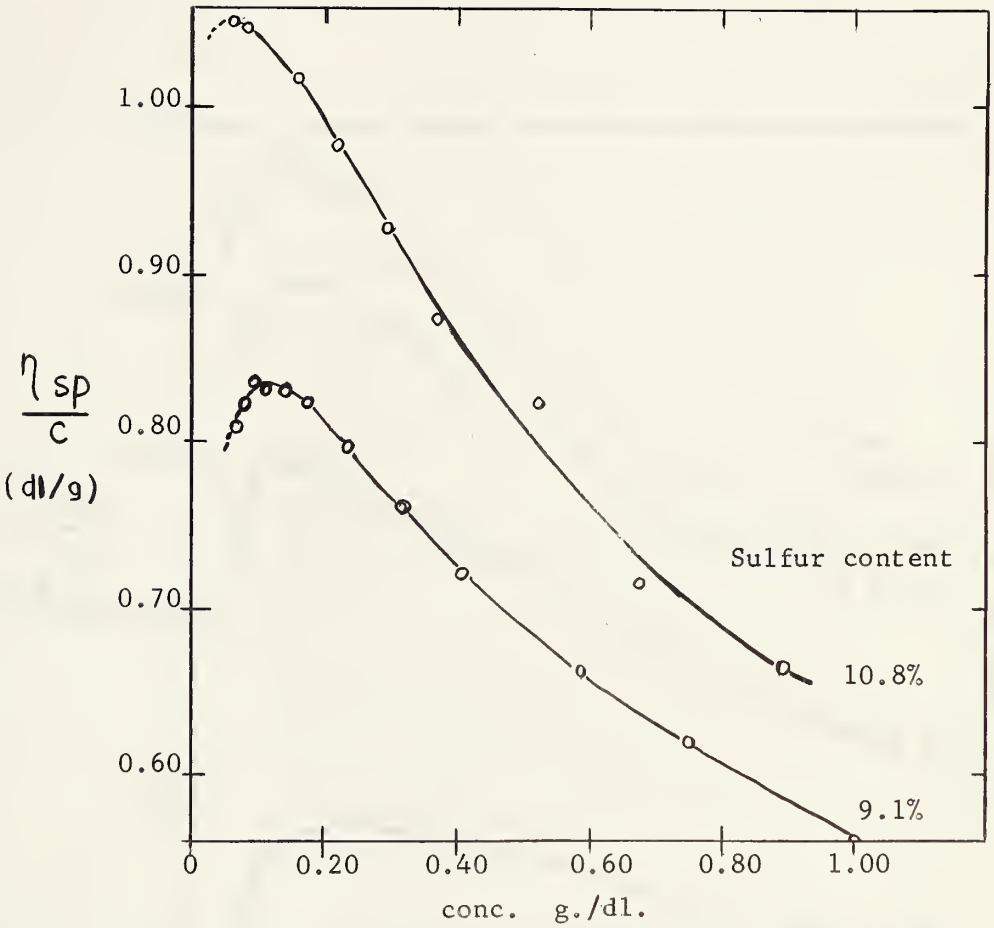


Figure 28. Plots of reduced viscosity vs concentration for linear potassium dextran sulfates in N/1000 KCl.

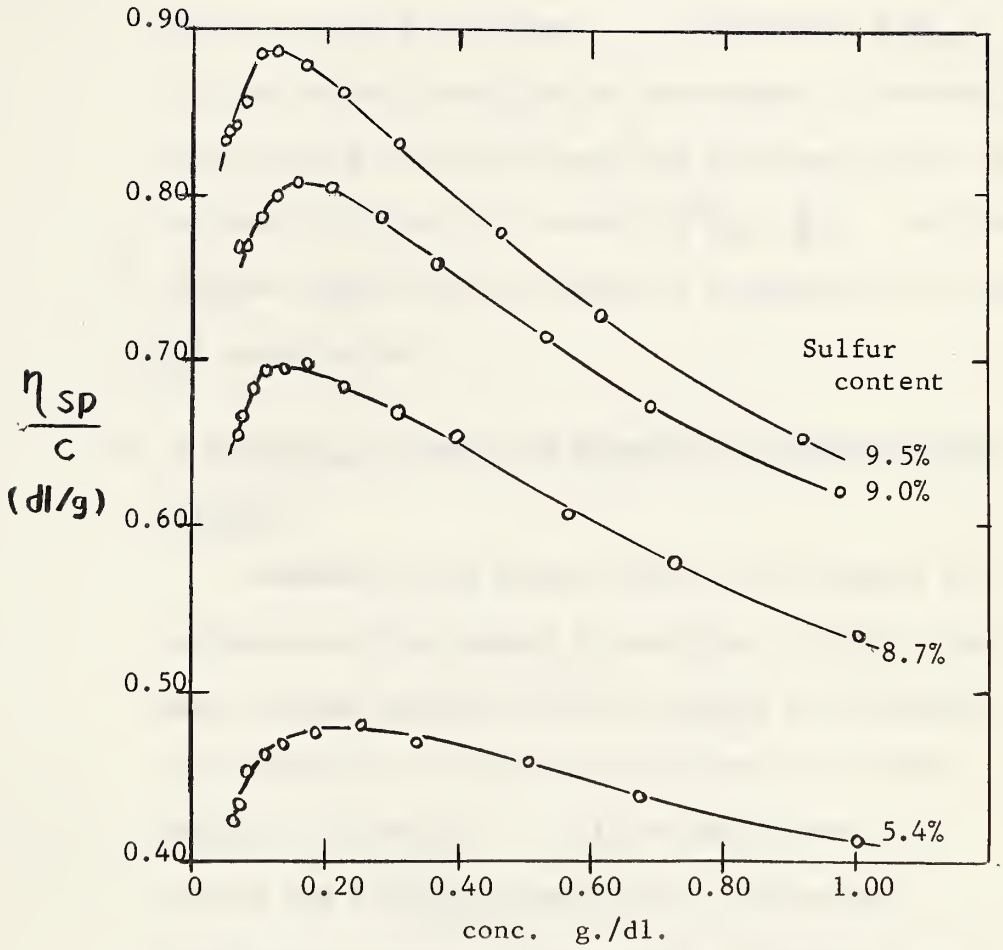


Figure 29. Plots of reduced viscosity vs concentration for branched potassium dextran sulfates in N/1000 KCl.

way that the curves form a "family" of curves.

The reduced viscosity at the maximum, $\left(\eta_{sp}/c\right)_{c_m}$ is of special interest. As the degree of substitution is increased, it shifts "to the left"; that is, the concentration at the maximum, c_m, decreased slightly with increasing sulfation. The value of $\left(\eta_{sp}/c\right)_{c_m}$ is particularly sensitive to the degree of substitution. This is true for both linear and branched species as is evident in Figure 30, in which $\left(\eta_{sp}/c\right)_{c_m}$ is plotted against percent sulfur (which is a measure of the degree of substitution).

(c) Comparison of Linear and Branched Potassium Dextran Sulfate

Because of the marked effect of the degree of sulfation on the reduced viscosities of both linear and branched dextran sulfate, samples for comparison were selected with as nearly as possible the same degree of sulfation: a linear sample with 9.1% sulfur and a branched sample with 9.0% sulfur. The reduced viscosity-concentration curves of these two samples, in N/1000 KCl, are shown in Figure 31. Though each of these curves belongs to a family of curves (see Figures 28 and 29), they obviously do

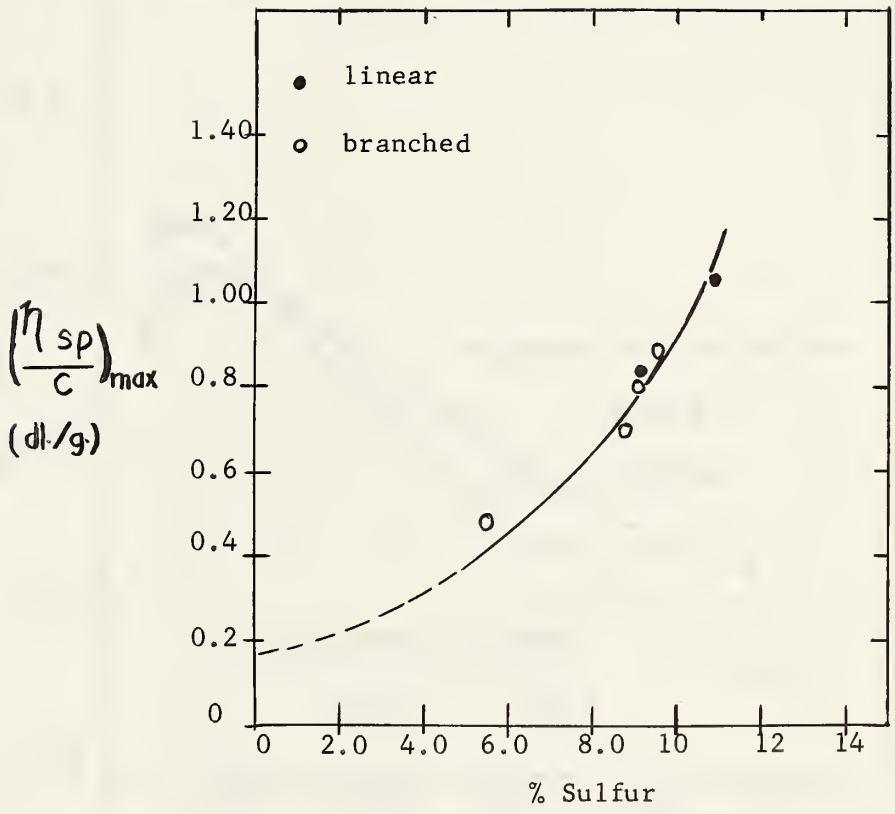


Figure 30. Plot of maximum value of $(\eta_{sp}/c)_{c_m}$ vs percent sulfur for potassium dextran sulfates in N/1000 KCl.

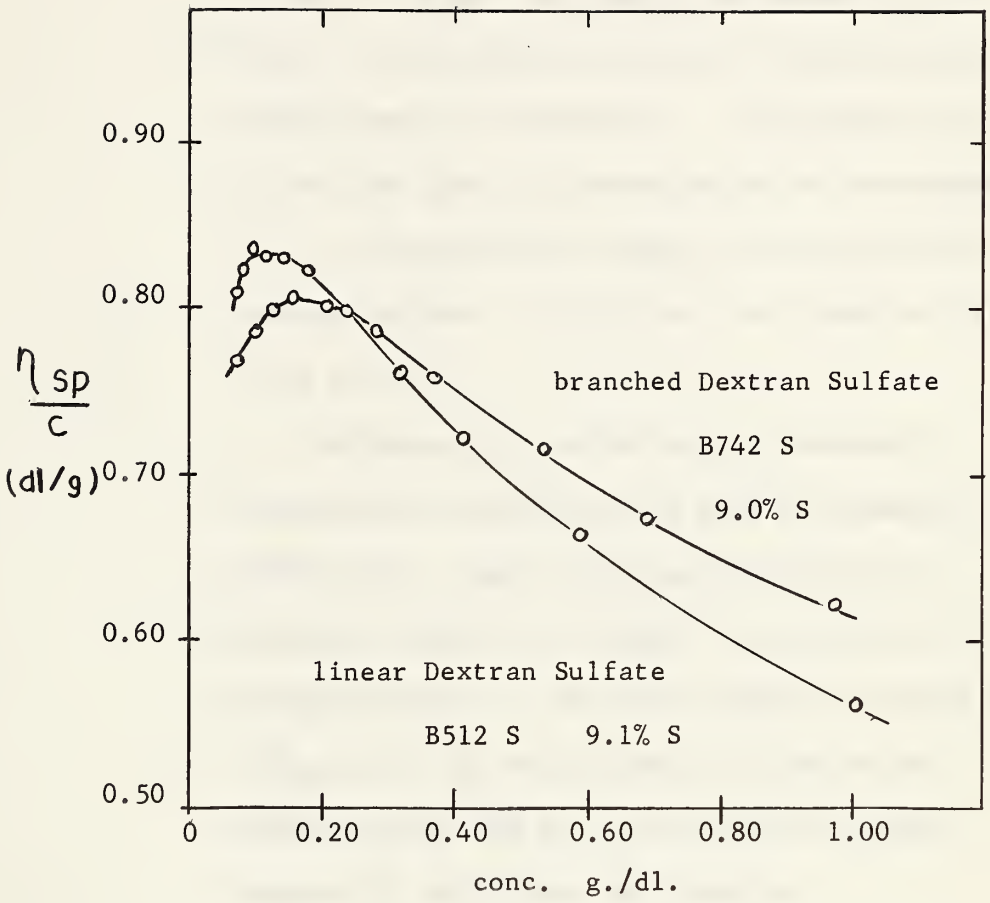


Figure 31. Plots of reduced viscosity vs concentration for branched and linear potassium dextran sulfates in N/1000 KCl.

not belong to the same family. Although the reduced viscosity at the maximum is lower for the branched than for the linear sample, the values of η_{sp}/c at higher concentrations are higher for the branched sample: the curves exhibit a marked "cross-over". Significant, too, is the fact that the concentration at the maximum, c_m, is significantly higher for the branched dextran sulfate: 0.165 g./dl. as compared with 0.115 g./dl.

In Figure 32 are the reduced viscosity-concentration curves for the same two samples in N/2000 KCl. Again the cross-over effect is apparent; again c_m is higher for the branched polyelectrolyte. In these curves the values of η_{sp}/c are all much higher than the values measured in N/1000 KCl, but in other respects Figures 31 and 32 are very similar.

In both, the maximum reduced viscosity $(\eta_{sp}/c)_{c_m}$ is lower for the branched dextran sulfate than for the linear dextran sulfate. At their respective maxima in the η_{sp}/c vs c plot, the branched and linear macromolecules are at their maximum extension for this particular

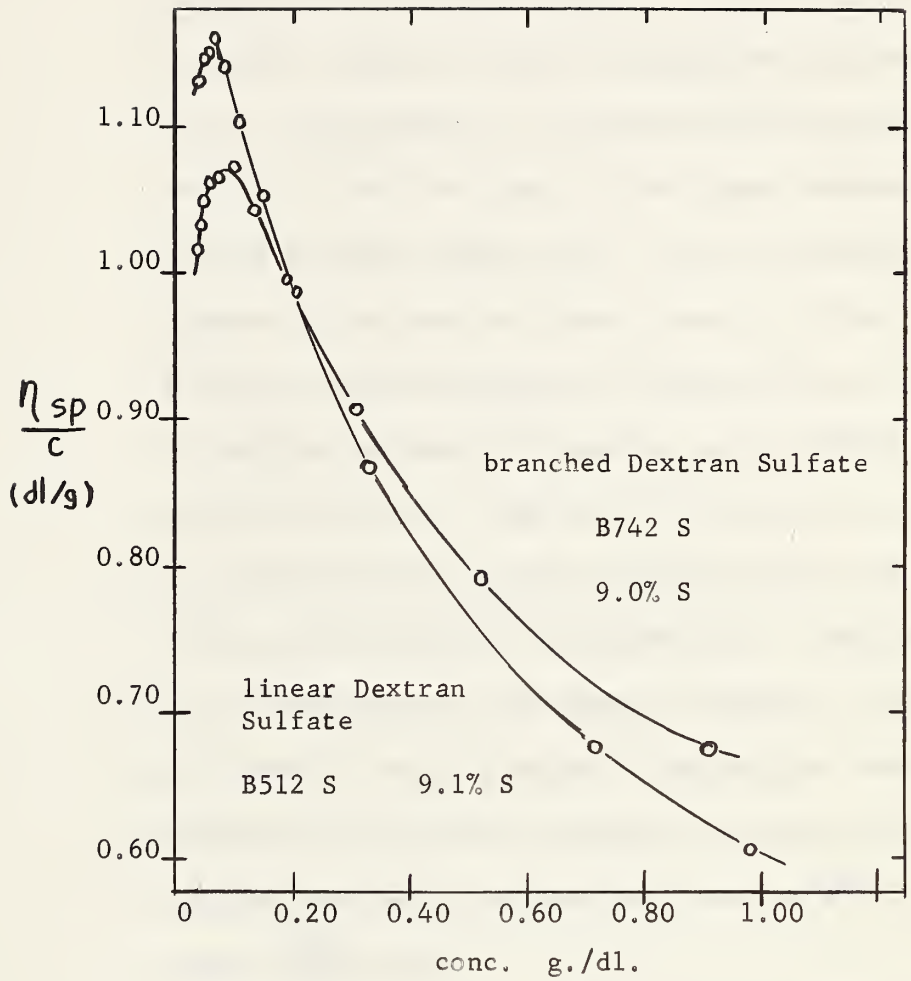


Figure 32. Plots of reduced viscosity vs concentration for branched and linear potassium dextran sulfates in N/2000 KCl.

solvent. It is to be expected that any difference in the viscosities of the parent polymers might manifest itself at this point. The intrinsic viscosity (and the reduced viscosity at a given finite concentration) of branched unsubstituted dextran is less than that of the linear unsubstituted dextran by about 0.007 dl./g.; for the branched dextran sulfate and the linear dextran sulfate in N/1000 KCl, the difference in reduced viscosity at the maximum is about 0.03 units, and in N/2000 KCl about 0.09 units. The small difference in sulfate content would account for less than a third of the peak difference in N/1000 KCl. The rest of the difference at the maxima, therefore, may be attributed to magnification of the initial small difference in viscosity between the linear and branched unsubstituted dextran (branched $[\eta]$ 0.157, linear $[\eta]$ 0.164).

The larger values of $(\eta_{sp}/c)_{cm}$ for the linear dextran sulfate is therefore to be expected; but the fact that at higher concentrations its η_{sp}/c values are smaller is not. The cross-over cannot be attributed to the (very slight) difference in degree of substitution for the curves

for samples of different degrees of substitution do not cross: they form a family. Similarly the cross-over cannot be due to differences in molecular weight (or molecular weight distribution) for as will be shown in Section D V c (and Figure 35) curves for dextran polyelectrolytes differing only in molecular weight also form a family.

The cross-over must therefore be due to the only other difference between the two samples -- the degree of branching.

V. Viscosity Behaviour of Carboxymethyl Dextrans

In order to determine whether the viscosity behaviour observed with the dextran sulfates, and particularly the cross-over phenomenon, would be observed also with another dextran polyelectrolyte, the same samples of branched and linear dextran as used in the preparation of dextran sulfates (as well as some linear samples differing in molecular weight) were carboxymethylated (as described in Section C III d) and their reduced viscosities were studied as a function of polyelectrolyte concentration in N/1000 KCl and N/2000 KCl.

(a) Effect of Shear Rate

As with the dextran sulfates (Section D IV a) a preliminary study was made of the shear rate dependence of the reduced viscosities.

Solutions of a branched carboxymethyl dextran (0.21 carboxymethyl groups per anhydroglucose unit; $\bar{M}_w$ 6.1 x 10^4) were prepared: (a) 0.1% in water (b) 0.1% in N/1000 KCl (c) 0.5% in water. Relative viscosities of these solutions are plotted against shear rate in Figure 27. There is no evidence of any shear dependence.

(b) Comparison of Linear and Branched Potassium Carboxymethyl Dextran

The reduced viscosity-concentration curves for the linear and branched samples of low degrees of substitution (in the range 0.06 to 0.21 carboxymethyl groups per A.G.U.) in N/2000 KCl are shown in Figure 33. No pair of curves exhibits the cross-over observed with the linear-branched pairs of dextran sulfates. The reduced viscosities of the linear member of the pair is lower throughout the entire concentration range study. There is, however, a slight indication at the highest degree of substitution that the curve for the

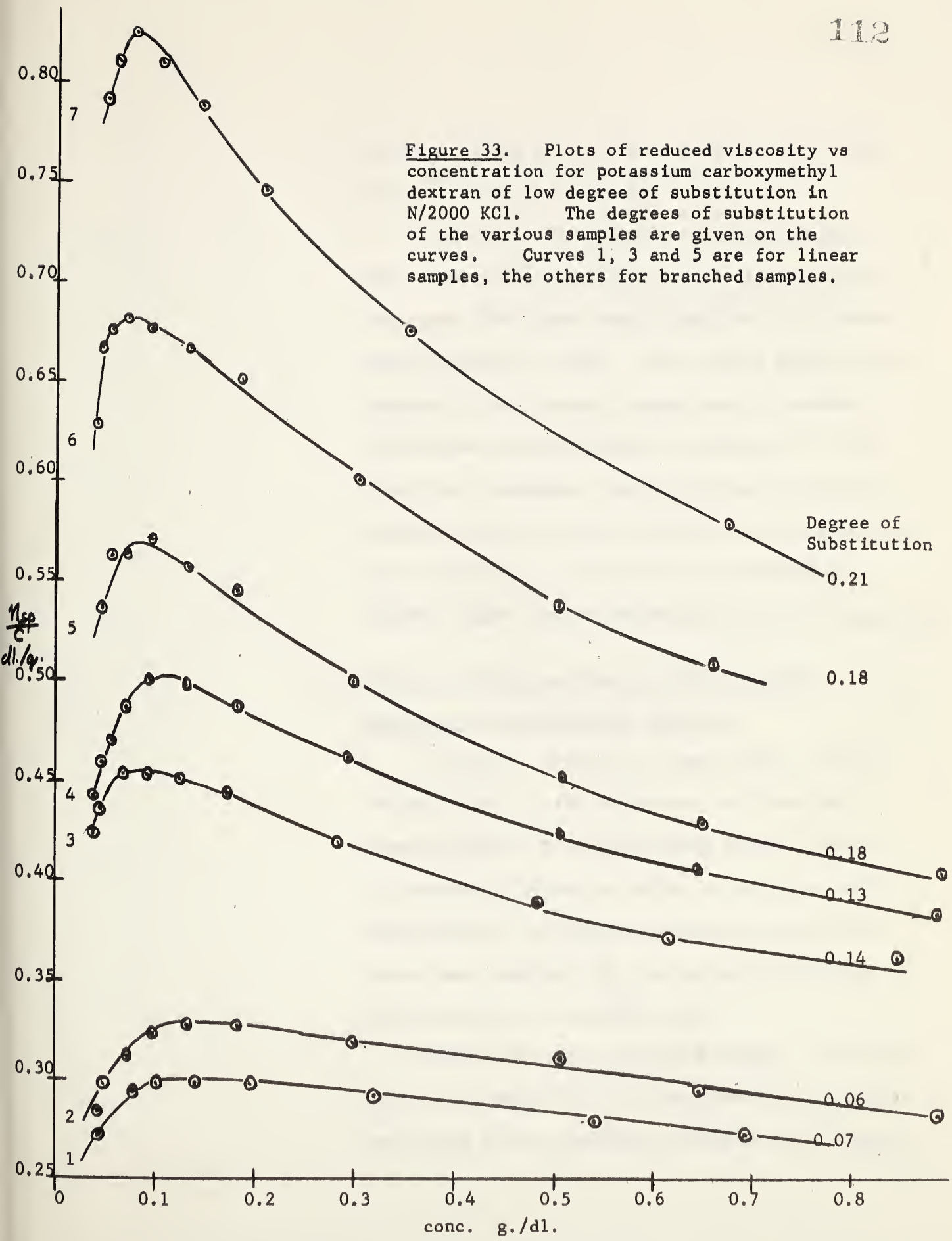


Figure 33. Plots of reduced viscosity vs concentration for potassium carboxymethyl dextran of low degree of substitution in N/2000 KCl. The degrees of substitution of the various samples are given on the curves. Curves 1, 3 and 5 are for linear samples, the others for branched samples.

branched sample does not belong to the same family as the curves for the linear samples.

Differences in behaviour were unmistakable when samples of much higher degree of substitution were used (the linear sample contained 1.22 carboxymethyl groups per A.G.U., the branched sample 1.14). Because of their higher charge density, reduced viscosities were determined in N/1000 KCl. The cross-over phenomenon which was observed with the dextran sulfates is also observed with these carboxymethyl dextrans -- the cross-over occurring at a somewhat higher concentration, $\approx$1.0 g./dl. (Figure 34).

(c) Effect of Molecular Weight on the Viscosity Behaviour of Carboxymethyl Dextrans

In order to discover to what extent, if any, the difference in the behaviours of linear and branched dextran polyelectrolytes might be due to differences in molecular weight or molecular weight distributions, the reduced viscosity-concentration curves were obtained for carboxymethyl dextrans differing only in molecular weight.

Linear dextrans of molecular weight 1.35×10^5, 1.194×10^5 and 7.31×10^4 (determined in the ultracentrifuge by the Archibald procedure) were carboxy-

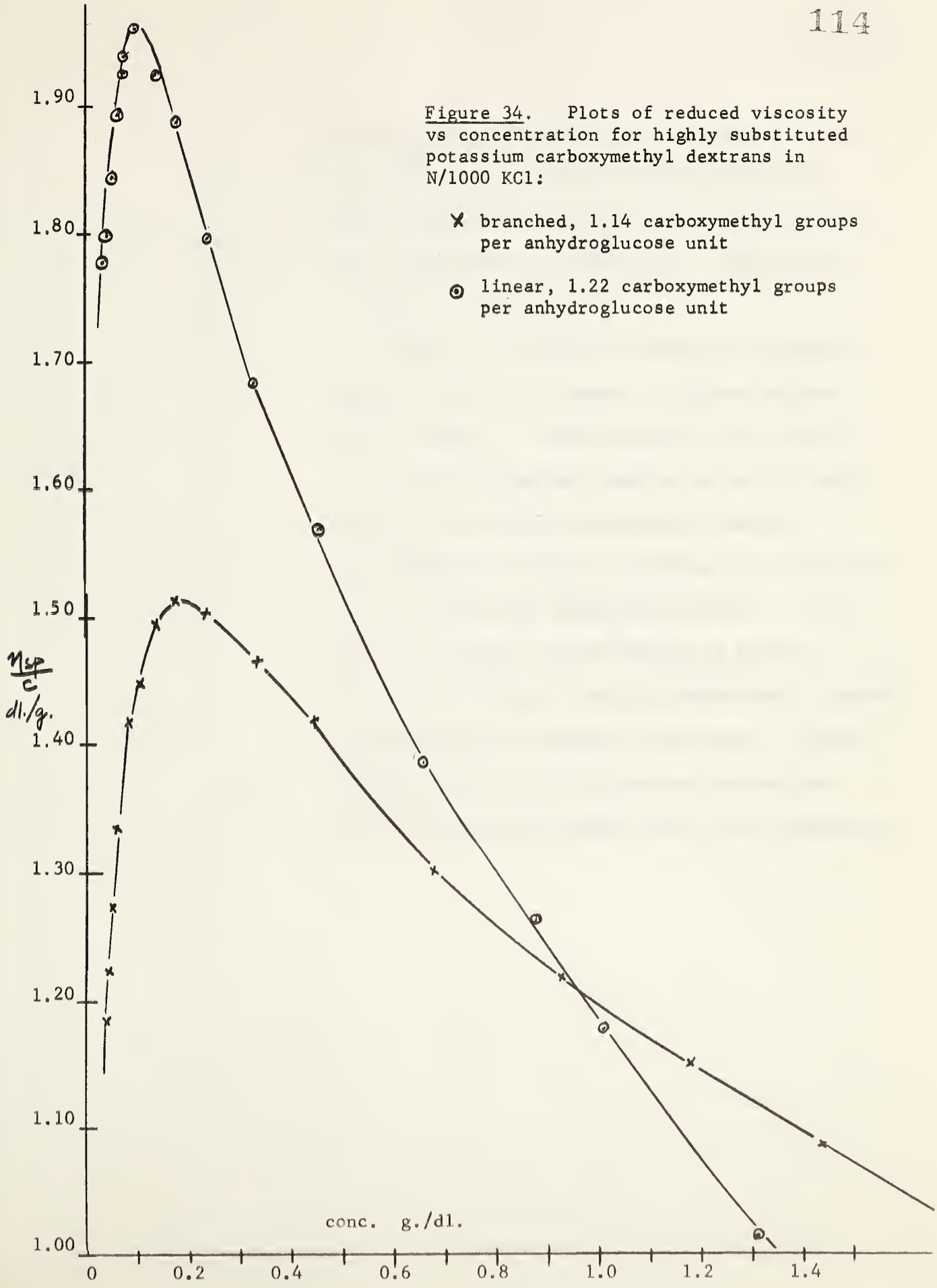


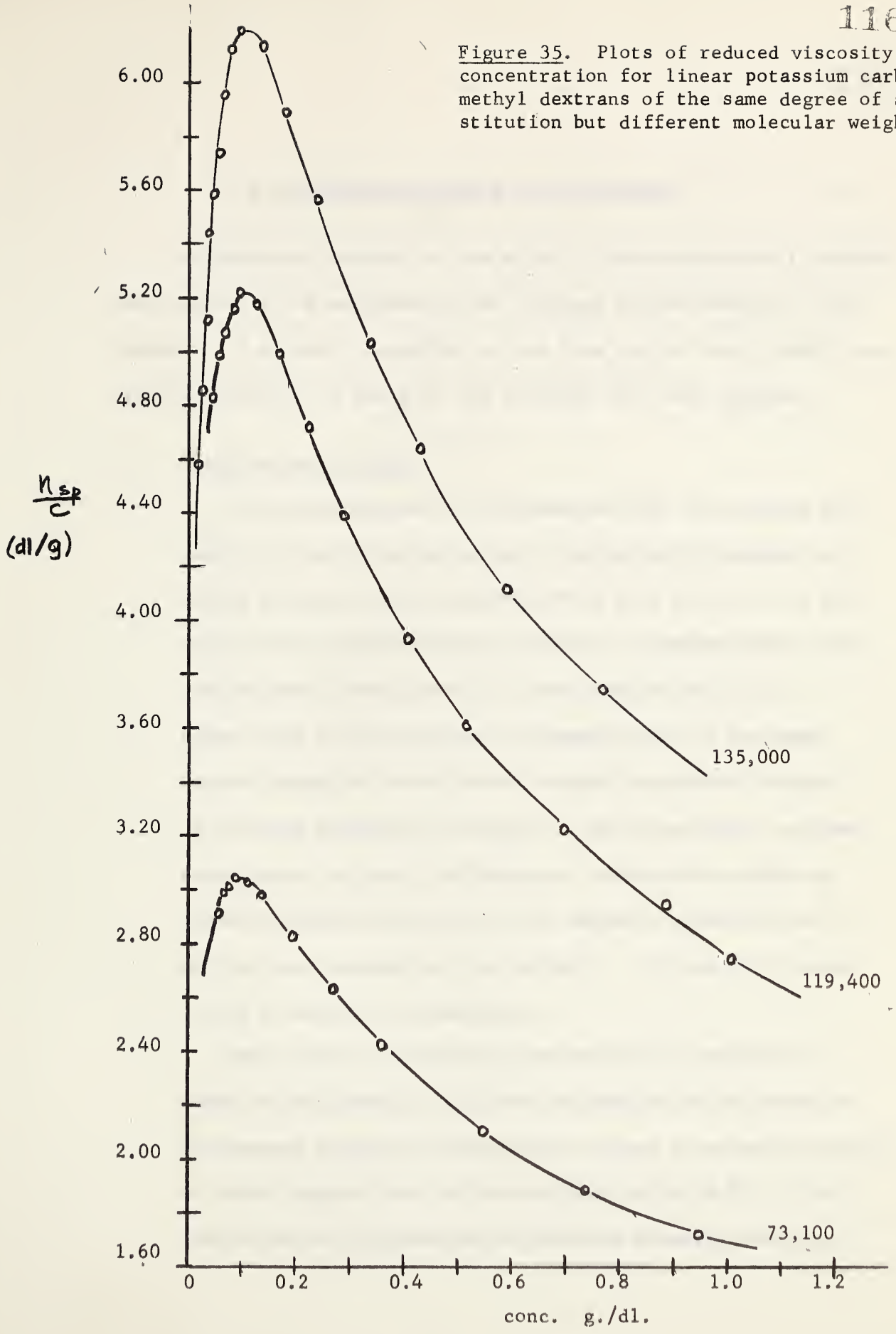
Figure 34. Plots of reduced viscosity vs concentration for highly substituted potassium carboxymethyl dextrans in N/1000 KCl:

- ✕ branched, 1.14 carboxymethyl groups per anhydroglucose unit
- ⊙ linear, 1.22 carboxymethyl groups per anhydroglucose unit

methylated to essentially the same degree of substitution: 0.82, 0.80, and 0.82 respectively. Their potassium salts were used for reduced viscosity measurements in N/1000 KCl. The results are shown in Figure 35.

Clearly, the curves all belong to the same family. Each curve keeps its proper distance from the other. The cross-over of the curves for linear and branched samples, noted with both dextran sulfates and carboxymethyl dextrans, cannot therefore be due to differences in molecular weight or molecular weight distribution. Supporting this fact are the results of Section D III which indicate that the sedimentation constant distributions are essentially the same -- no gross difference in distribution existed between the linear and branched samples used in the comparisons.

Figure 35. Plots of reduced viscosity vs concentration for linear potassium carboxymethyl dextrans of the same degree of substitution but different molecular weights.



E. GENERAL DISCUSSION AND CONCLUSION

Information obtained in the study of the carboxymethyl dextrans both confirms and supplements the findings of the dextran sulfate study. A general discussion of the data and of their significance should therefore be based on the findings with both systems.

I. The Cross-over Effect

With both systems it was observed that the reduced viscosity vs concentration curves, obtained with branched and linear polyelectrolyte samples (which were derived from dextrans of the same intrinsic viscosity) of approximately the same degree of substitution in the same solvent (i.e., aqueous KCl of the same ionic strength) were of the same general shape but nevertheless crossed one another because of a marked difference in slope. The cross-over, we have seen, cannot be due to differences in molecular weight or molecular weight distribution, in degree of substitution, or in the ionic strength of the solvent. It can only be due to the difference in branching.

This effect of branching can readily be explained in terms of the generally accepted explanation of the shape of the reduced viscosity-concentration curves of polyelectrolytes in dilute aqueous salt solutions (see Section B IV). The charges on the polyelectrolyte molecule mutually repel one

another and the molecule expands until this expansion tendency is balanced by the factors favoring contraction. The greater the charged density on the molecule, the greater the expansion. With dilution, the number of charged sites on the molecule increases by displacement of the equilibrium

$$(PX^{-}M^{+}) \rightleftharpoons PX^{-} + M^{+}$$

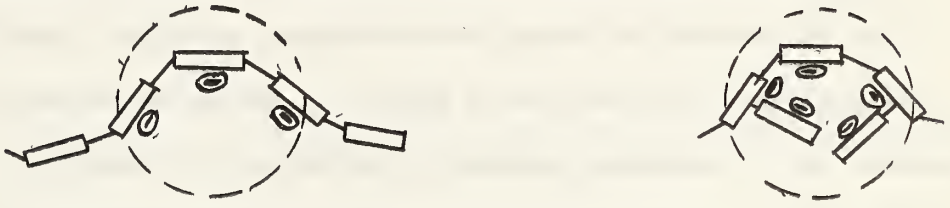
so long as the concentration of positive ions in the solvent is less than the concentration in the immediate neighborhood of the charged molecule.

The parent linear dextran has a slightly larger intrinsic viscosity (0.164) than the parent branched dextran (0.157) and a slightly larger reduced viscosity at c = 1.0 g./dl. (0.176 compared with 0.170). Hence in the same volume of solution at the same concentration the linear dextran molecules will have a slightly larger volume than the branched ones. On the average the contour length of the linear dextran molecule would be of the order of 240 anhydroglucose units (A.G.U.) since the molecular weight is 40,000, whereas the contour length of the branched molecule ($\bar{M}_w$ 61,000) would be less than this: 148 to 175 A.G.U. (assuming average branch length of 2 to 1.5 A.G.U.).

Consider now the consequences of (a) introducing ionogenic sites -- the same number per gram of macromolecule --

onto the linear and branched molecules in solution, and (b) subsequent dilution of the solutions.

Because of the branching, the local charge density will be higher in the branched than in the linear molecules



and therefore the expansion of the branched molecules will be greater. Consequently in the polyelectrolyte solution, η_{sp}/c for the branched polyelectrolyte will be greater than for the linear one at the same concentration, as the branched molecules will have done more of the expanding possible to them. As the solutions are diluted, both kinds of molecules expand further. But, ultimately, the linear molecules should occupy the larger volume, partly because of their greater (average) contour length, partly because they would be less sterically hindered in achieving full extension. Thus at the high concentration η_{sp}/c would be greater for the branched polyelectrolyte than for the linear one, but with sufficient dilution the η_{sp}/c would become greater for the linear polyelectrolytes.

One can also explain why no cross-over is observed when the degree of substitution is much lower (as with the lightly

substituted carboxymethyl dextrans). The charges would be scattered much more thinly along the molecules; but in the coiled branched molecules the overall charge density would still be greater than in the coiled linear molecules. Hence, relative expansion would again be greater for the branched molecules. (This effect would presumably also contribute to the initially greater expansion of the branched highly substituted dextran polyelectrolytes.) With the lightly charged polyelectrolytes, however, the maximum expansion achievable as a result of dilution would be much smaller for both branched and linear molecules and would be achieved when the linear molecules were still relatively less expanded than the branched ones.

The cross-over with the carboxymethyl dextrans (the highly substituted ones) occurs at a much higher concentration than that of the dextran sulfates. This observation is consistent with our explanation. The carboxymethyl dextrans that exhibited no cross-over had a very low degree of substitution (in the range 0.06 -- 0.2). The dextran sulfates had a degree of substitution of 0.7 -- and the cross-over occurred close to the maxima of the curve. The highly substituted CMD's had a degree of substitution greater still -- 1.2 -- and the cross-over occurred further from the maxima. The higher the charge, the greater the ratio of c_{cr} to c_m . This

is (when c_{cr} is the concentration at the cross-over) true even when the higher charge is achieved by using a solvent of lower ionic strength. For example, for the branched dextran sulfate (DS = 0.68) the ratio c_{cr} to c_m is 1.48 in N/1000 KCl and 2.24 in N/2000 KCl.

II. Characteristic Features of the Reduced Viscosity-Concentration Curves of the Dextran Polyelectrolytes.

The cross-over effect has been shown to be due to differences in degree of branching, which result in differences in η_{sp}/c vs c curves. These differences may be revealed in another way, by focussing attention on certain characteristic features of these curves, namely: the reduced viscosity at the maximum, $\left(\eta_{sp}/c\right)_{c_m}$; the concentration at the maximum, c_m; and the "shape" of the curves with particular reference to the slope of the curves on the high-concentration side of the maximum. The relation between these and such features as molecular weight, degree of substitution and branching of the polyelectrolyte and the ionic strength of the solvent will be briefly considered.

(a) Maximum Reduced Viscosity $\left(\eta_{sp}/c\right)_{c_m}$

As might be expected the maximum reduced viscosity increases with increasing molecular weight (see Figure 35). (The three samples of carboxymethyl dextran were

substituted to the same degree and the measurements made in the same solvent at the same temperature, 25°C.) A log-log plot -- Figure 36 -- indicates that the maximum reduced viscosity is related to the molecular weight by the equation

$$\left(\eta_{sp/c}\right)_{c_m} = KM^{1.1}$$

which is reminiscent of Staudinger's early equation for polymers (low molecular weight)

$$\left(\eta_{sp/c}\right) = KM$$

The value of the maximum reduced viscosity increases with degree of substitution -- Figure 29 -- and decreases with increasing ionic strength of the solvent -- Figures 31 and 32. Both of these effects are to be expected, for both increasing the degree of substitution and decreasing the ionic strength of the solvent increases the charge density on the polyelectrolyte molecules. The nature of the charged group also effects the values of the maximum reduced viscosity. In Figure 37 the maximum reduced viscosity is greater for the carboxymethyl dextran than for a dextran sulfate of higher degree of substitution. This suggests that ion binding is considerably weaker for the carboxymethyl group ($-CH_2COO^-K^+$) than for the sulfate

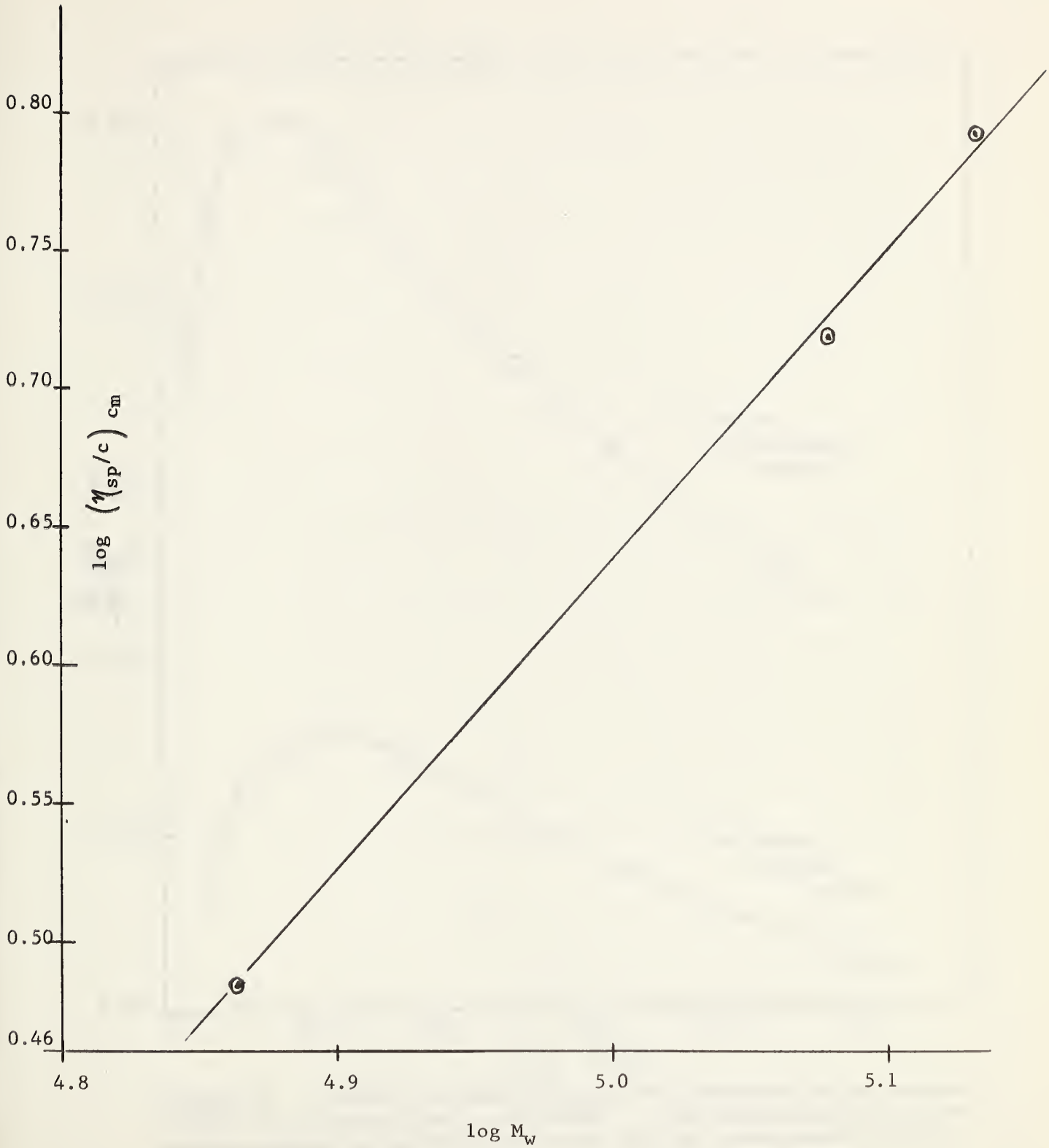


Figure 36. Logarithmic plot of $(\eta_{sp}/c)_{c_m}$ of carboxymethyl dextran vs the molecular weight of the unsubstituted dextran.

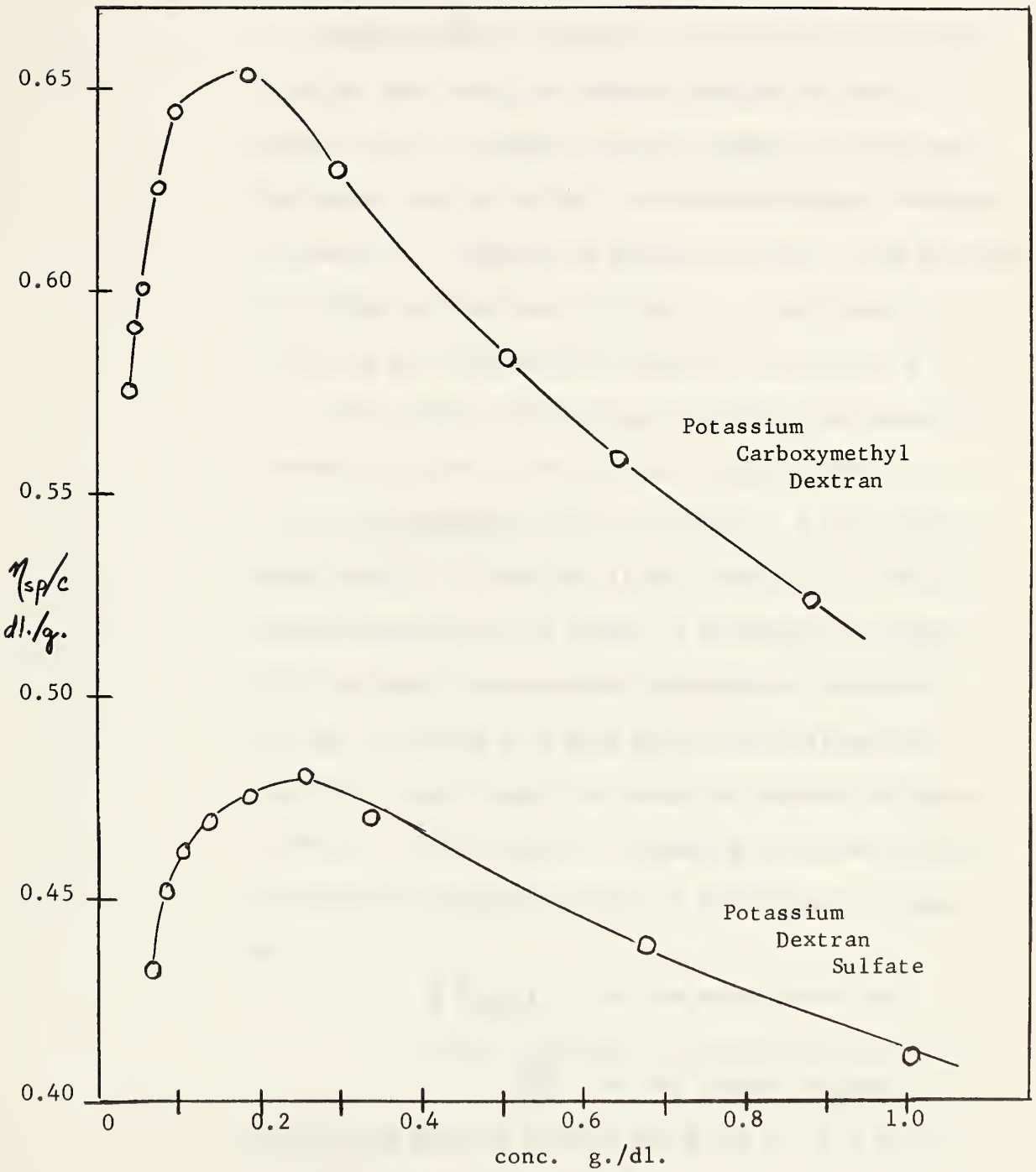


Figure 37. Plots of reduced viscosity vs concentration of branched dextran polyelectrolytes in N/1000 KCl. The sulfate contains 0.34 sulfate groups per anhydroglucose unit and the carboxymethyl dextran contains 0.21 carboxymethyl groups per anhydroglucose unit.

group ($-SO_2O^-K^+$).

Branching must also have a large effect for despite the fact that the branched dextran sulfate in Figure 31 has a larger molecular weight (61,000) than the linear sample (40,000) its maximum reduced viscosity is smaller. (Degree of substitution and ionic strength of solvent are the same for both.) The effect of branching has already been explained in Section E I.

This effect of branching is greatly increased by comparing values of the maximum reduced viscosity for linear and branched polyelectrolytes at a high degree of substitution. With the lightly substituted carboxymethyl dextrans little effect of branching is evident. With the highly substituted carboxymethyl dextrans (DS ca 1.2) there is a very large effect (Figures 33 and 34) -- much larger than with the dextran sulfates of DS 0.7. One method of comparing the effect quantitatively is to compare values of the expansion factor, F:

$$F = \frac{\left(\eta_{sp}/c\right)_{c_m} \text{ for the polyelectrolyte}}{[\eta] \text{ for the parent polymer}}$$

In branched dextran sulfate (DS 0.68) F = 5.1 and for linear dextran sulfate (DS 0.69) F = 5.1 (both in

N/1000 KCl). By comparison, for branched carboxymethyl dextran (DS 1.14) F = 9.65 and for linear carboxymethyl dextran (DS 1.22) F = 12.07. Even after correcting for the larger degree of substitution of the linear sample (i.e., correcting to a value of DS of 1.14) the value of F for linear carboxymethyl dextran is still much larger -- 11.30 -- than that for the branched sample. At high degree of substitution, then, the effect of branching on the maximum reduced viscosity is magnified.

(b) Polyelectrolyte Concentration c_m at which the Reduced Viscosity Maximum Appears.

The concentration at which the reduced viscosity maximum appears, c_m, is affected by most of the factors that influence the maximum reduced viscosity -- but not by all. Differences in molecular weight (same polymer system -- see Figure 35) have no effect on c_m. For linear samples of molecular weight 73,100; 119,400 and 135,000 -- same degree of substitution, same solvent -- the c_m value is 0.10 g./dl. Increasing the degree of substitution or decreasing the ionic strength of the diluting solvent does, however, cause a decrease in the value of c_m. For example, c_m for a branched dextran sulfate of degree of substitution 0.34

is 0.235 g./dl. and for one of 0.74 it is 0.125 g./dl. (see Figure 29); and for a linear dextran sulfate (DS 0.69) c_m changes from 0.115 to 0.063 g./dl. in going from N/1000 to N/2000 KCl (see Figures 31 and 32 and Table XXI).

Branching has a marked effect on the value of c_m as is evident from the data of Table XXI; in both solvents the branched polyelectrolyte has unmistakably the higher c_m (by factors of 1.46 and 1.42).

These effects also may be explained on the basis of molecular expansion because of mutual repulsion of charges. The ion binding at the charged groups decreases with dilution because of a shift of the equilibrium

$$(PX^-K^+) \rightleftharpoons PX^- + K^+$$

causing increased molecular expansion. Eventually a point is reached where the cation concentration within the domain of the macroion is nearly identical to that of the surrounding solution. This is the point at which η_{sp}/c is a maximum (and $c = c_m$); for with further dilution the cation concentration in solution becomes increasingly greater with respect to the cation concentration in the macroion domain, polyelectrolyte dissociation is increasingly suppressed, the charge density in

the molecule decreases and η_{sp}/c falls. So, the higher the density of charge sites on the molecule and the lower the concentration of cations in the diluting solvent, the further will dilution have to proceed to reach the balance of effects at the maximum (and hence the lower will c_m be).

The effect of branching on c_m is probably due to the greater local charge density in the branched molecule. This would result in a smaller decrease in the ion binding with dilution (smaller shift "to the right" of the equilibrium $(PX^-K^+) \rightleftharpoons PX^- + K^+$), and the maximum would occur at higher concentration of polyelectrolyte.

(c) Shape of Curve

Attention has already been called to the close family relationships in the various sets of curves of Figures 28, 29, 33 and 35.

This family relationship is at its simplest in the curves for samples differing only in molecular weight (see Figure 35). Each curve of the set can be reduced to any other -- say a to b -- simply by multiplying each value of η_{sp}/c for a by the ratio $(\eta_{sp}/c)_b / (\eta_{sp}/c)_a$ at any given concentration. All the curves are the same except for an effect of molecular weight on η_{sp}/c,

which is the same at every concentration. Essentially, then, all these curves have the same shape. (If one curve were plotted on rubber sheet graph paper, the curve for a sample of higher molecular weight could be obtained by suitable uniform vertical stretching.) Such simple interconversion is, however, not possible with the other sets of curves, for which c_m is not a constant.

One means of comparing these curves is to calculate a factor related to the expansion of the molecules as the maximum expansion is approached with dilution: namely, the ratio of the maximum reduced viscosity $\left(\eta_{sp}/c\right)_{c_m}$ (which with different curves occurs at different c_m's) to the reduced viscosity at a concentration which is some simple multiple of c_m. This "relative expansion ratio" is, then, defined as

$$R_n = \frac{\left(\eta_{sp}/c\right)_{c_m}}{\left(\eta_{sp}/c\right)_{nc_m}}$$

where n equals 2, 3, 4 etc. Relative expansion ratios for a number of systems are given in Table XXII.

Close examination reveals some interesting relations between R_n at a given value of n, and the factors that affect $\left(\eta_{sp}/c\right)_{c_m}$ and c_m:

R_n is independent of molecular weight (compare a,b and c)

R_n increases with decreasing ionic strength of solvent (compare d and f, e and g)

R_n decreases with increasing degree of substitution for the sulfate system (compare d and h)

R_n increases with decreasing degree of substitution for the carboxymethyl dextran system (compare c and i)

R_n is independent of branching (compare d and e, f and g)

The fact that R_n is not affected by branching is certainly not apparent from an inspection of the η_{sp}/c curves (see Figure 31). It does suggest, however, a basic similarity in the molecular expansions which might be made more evident by plotting against a concentration suitably reduced, that is, against a concentration expressed as a function of c_m. Accordingly, in Figure 38, η_{sp}/c is plotted against c/c_m for linear and branched dextran sulfates of the same degree of sulfation (0.68 and 0.69). The two curves are parallel.

In the same figure is plotted the curve for dextran sulfate of higher degree of sulfation (DS 0.74). This curve is nearly parallel to the others. However, if an attempt is made to make these three curves coincide (comparable to that used successfully with the molecular weight family) by multiplying values of η_{sp}/c by the ratio of the $\left(\eta_{sp}/c\right)_{c_m}$ values, the results shown in

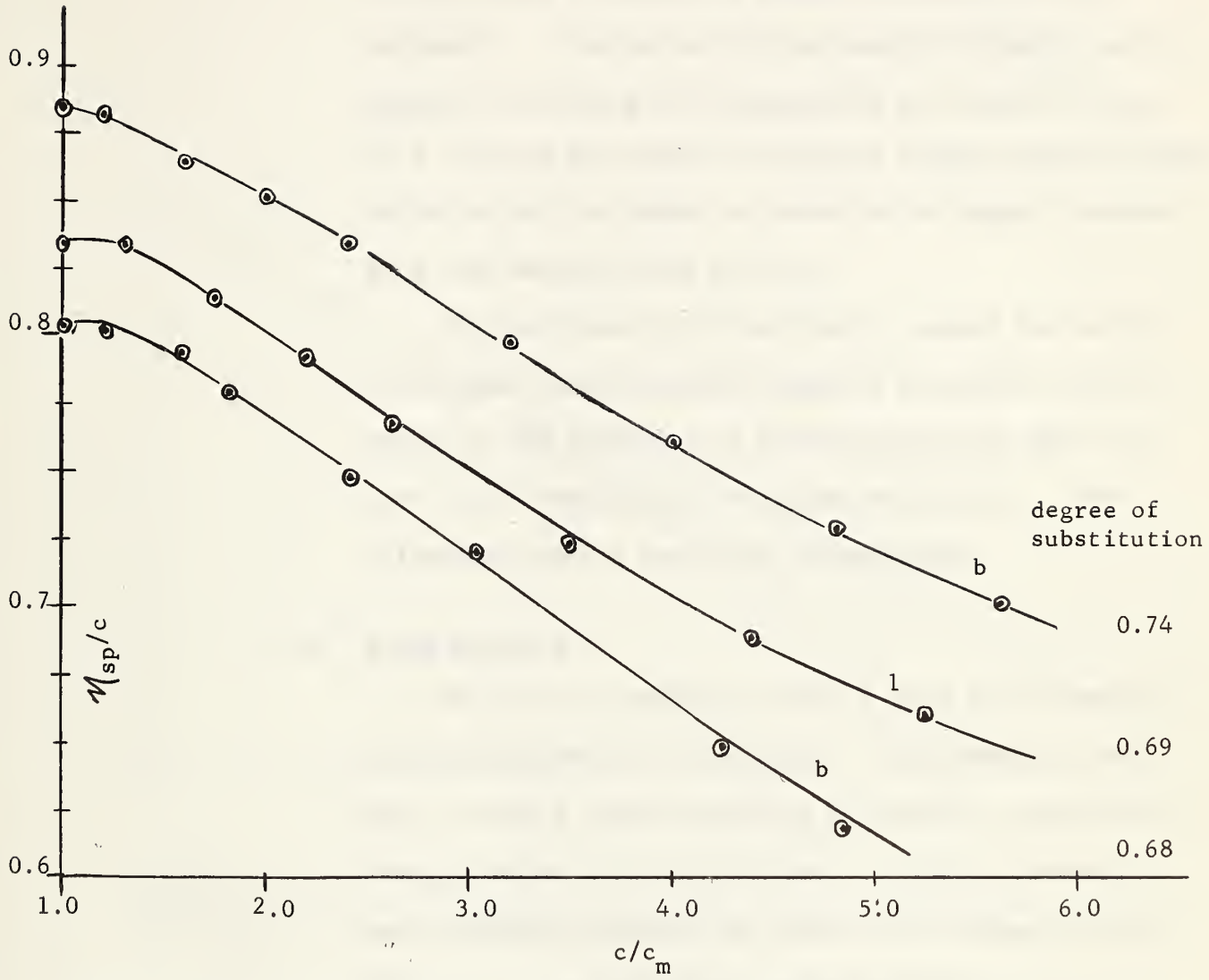


Figure 38. Plot of reduced viscosity vs the reduced concentration c/c_m for linear (l) and branched (b) dextran sulfates in N/1000 KCl.

Figure 39 are obtained. The curves coincide at values of c/c_m close to unity but steadily diverge as c/c_m increases. The curves for the sample differing only in degree of branching are identical up to a value of c/c_m of 3, whereas the curves for the two linear polyelectrolytes differing only in degree of substitution begin to diverge at a much smaller value of c/c_m.

The near identity of the doubly reduced curves for linear and branched species supports the belief that the nature of the expansion of branched molecules with dilution is the same as that of linear molecules. The differences are in the "rate" of expansion.

(d) Slope Factor b

The relative expansion ratio R_n does not, therefore, reveal differences in branching. Any parameter that does so should relate expansion to absolute concentration changes rather than relative ones. Such a parameter was obtained by plotting R_n values for different values of n -- i.e., at different concentrations $2c_m$, $3c_m$, $4c_m$ etc. -- against these concentrations expressed in absolute rather than relative units.

Such plots are shown in Figure 40. All give straight lines which satisfy the equation

$$R_n = 0.91 + bc$$

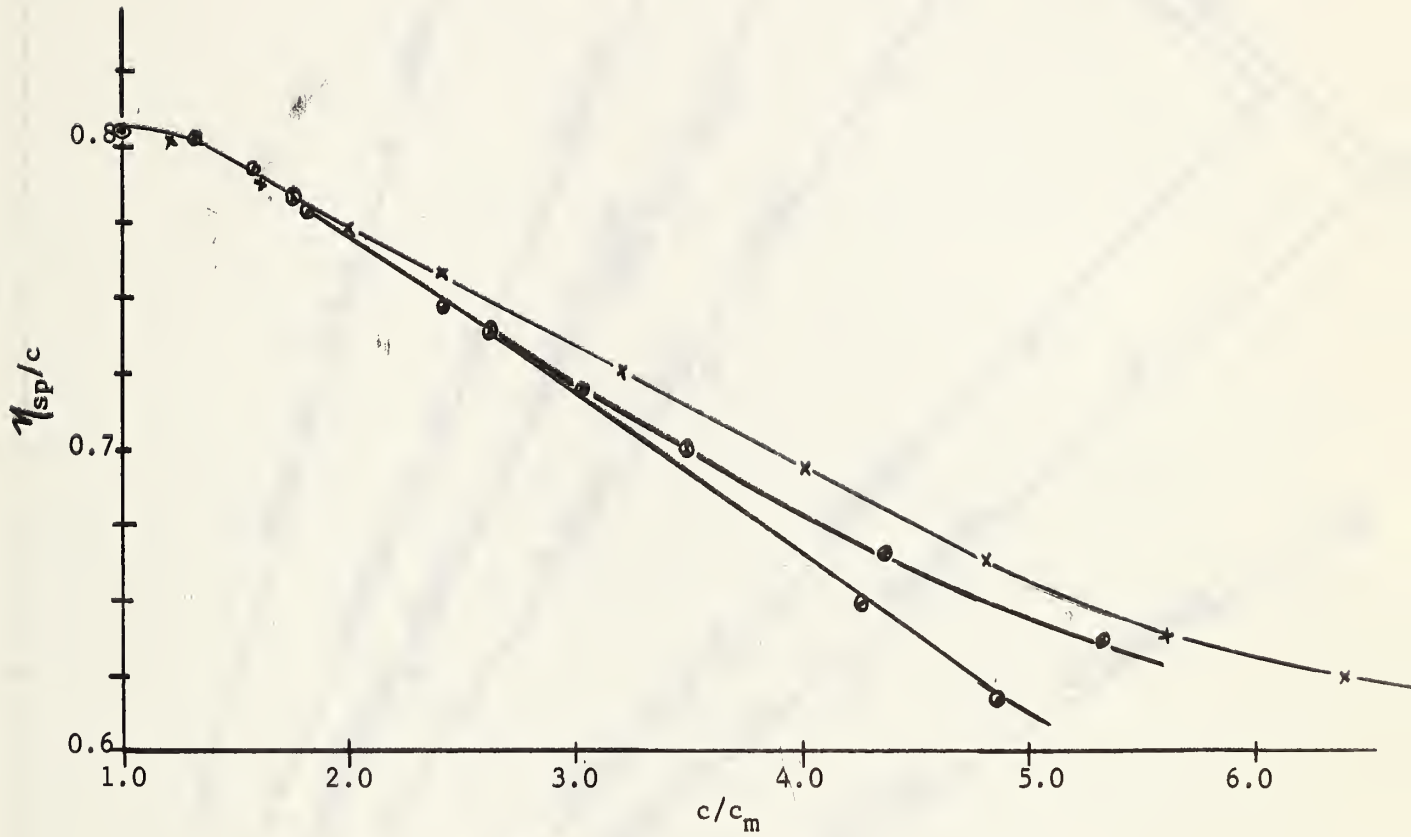


Figure 39. Curves of Figure 38 normalized by the application of a factor equal to the ratio of maximum reduced viscosities: -⊙- branched DS 0.68, -⊗- linear DS 0.69, -×- branched DS 0.74.

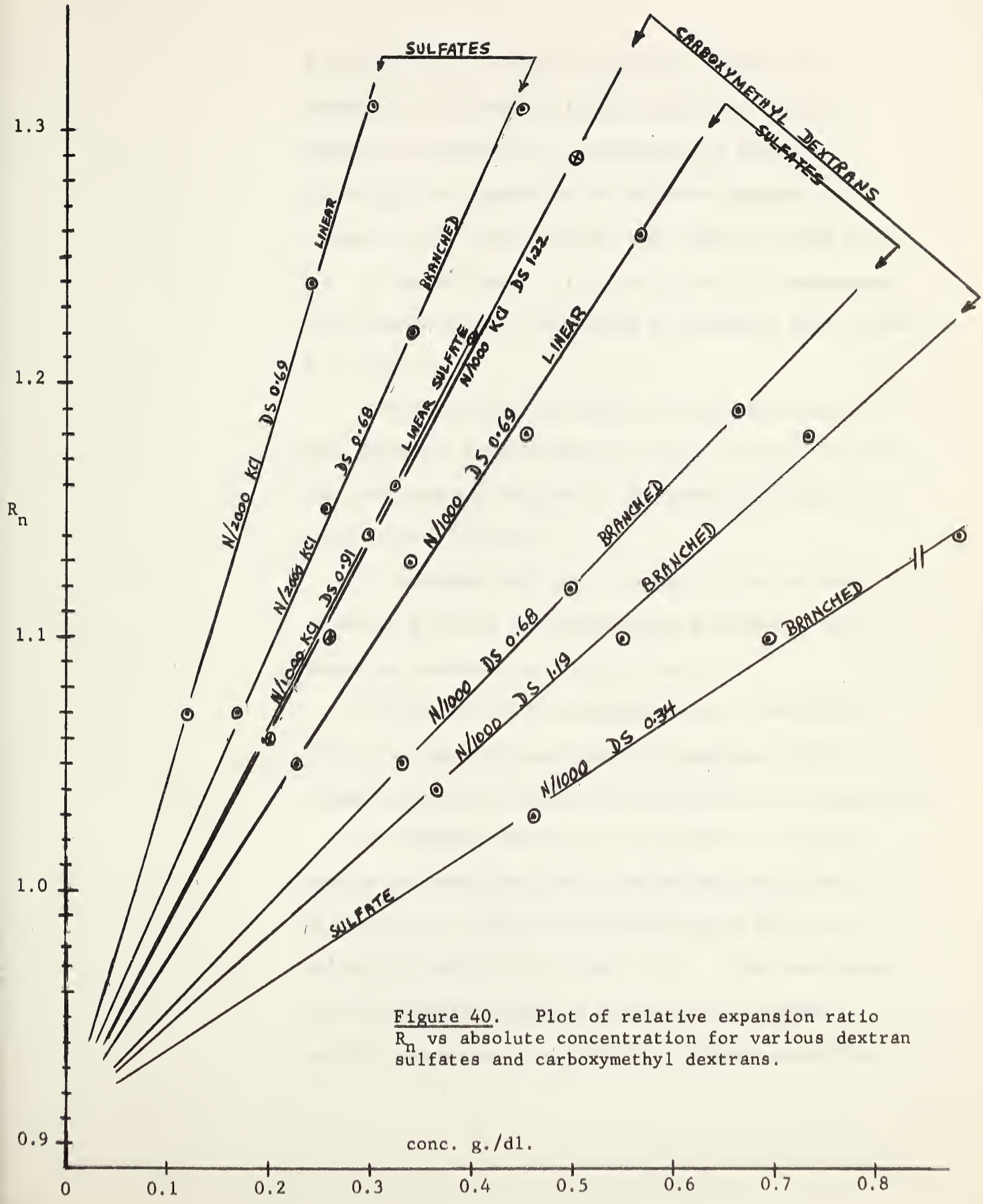


Figure 40. Plot of relative expansion ratio R_n vs absolute concentration for various dextran sulfates and carboxymethyl dextrans.

A similar plot for the three linear carboxymethyl dextrans of differing molecular weight but of same degree of substitution is reproduced in Figure 41. The significant parameter in the above equation is b. Values of this "slope factor" are listed in Table XXIII.

The slope factor b is (like c_m and R_n) independent of molecular weight (see Figure 41 and Table XXIII, items a, b and c).

With the dextran sulfates, it increases markedly with degree of substitution (d and e, f and g); but with the carboxymethyl dextrans it decreases with degree of substitution (c and j).

It increases with ionic strength of the solvent (d and h, g and i) and decreases very markedly, with degree of branching (d and g, h and i).

This factor has the advantage that it magnifies effects and permits quantitative comparisons which, though rough, are nevertheless interesting and suggestive.

For example, the ratio of b values for linear dextran sulfates (DS 0.69) in N/1000 KCl and N/2000 KCl is 0.65/1.30 = 0.50; and for the branched dextran sulfates this ratio is 0.41/0.88 = 0.47. The same change in ionic strength produces substantially the same relative change in b with the two different materials.

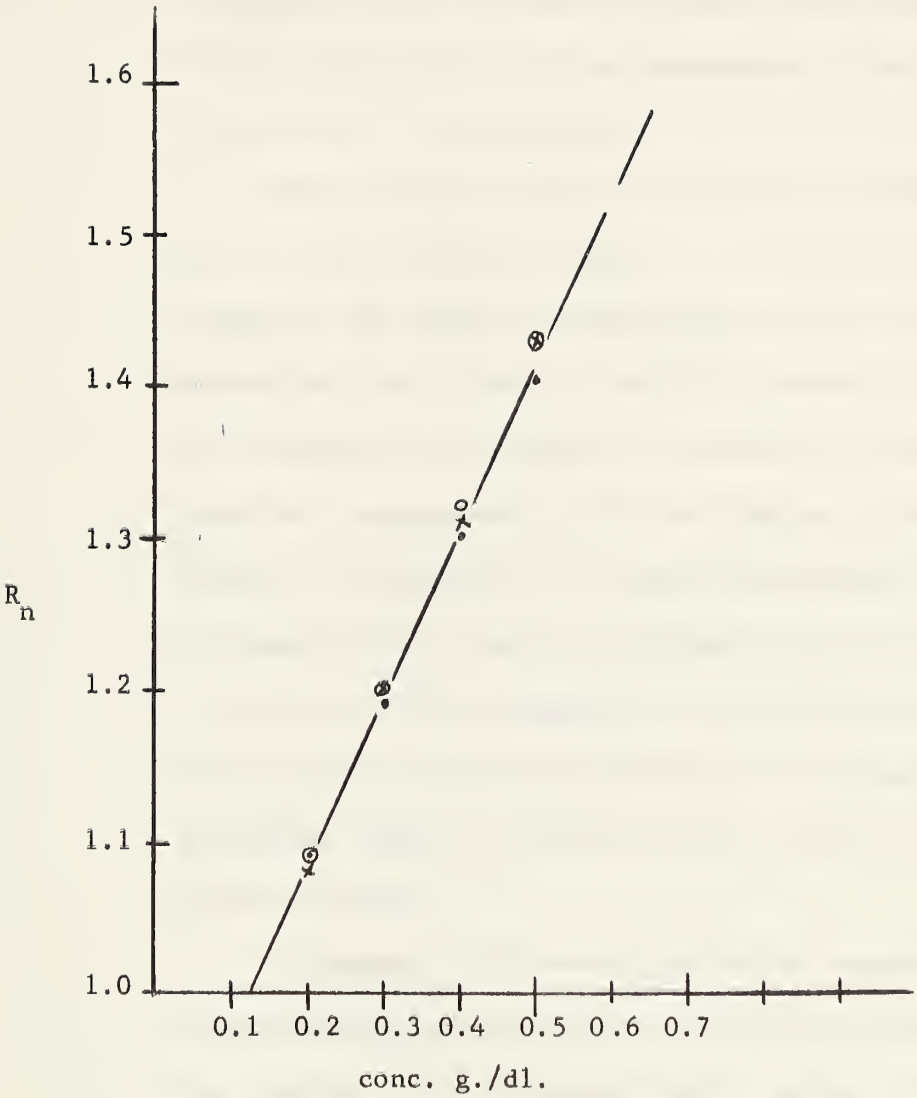


Figure 41. Plot of relative expansion ratio R_n vs absolute concentration for carboxymethyl dextrans of three different molecular weights.

- ⊙ 135,000 (DS 0.82)
- ✖ 119,400 (DS 0.80)
- • 73,100 (DS 0.82)

Again the ratio of b's for branched and linear dextran sulfates is 0.63 in N/1000 KCl and 0.68 in N/2000 KCl. Within the precision of the measurement, these ratios may be said to be the same.

The value of b varies with degree of substitution but not with molecular weight. If the relationship between b and degree of substitution were accurately determined over a range of degree of substitution values, then values of b obtained for branched and linear samples of approximately the same degree of substitution could be corrected for the slight differente in degree of substitution; then the difference in the corrected b values would be a measure of the degree of branching. In this way, a relationship between b and degree of branching might be established for a given polyelectrolyte-solvent system.

In summary, this investigation has demonstrated that branching affects three characteristic features of the reduced viscosity-concentration curves: the reduced viscosity at the maximum $\left(\eta_{sp}/c\right)_{c_m}$, the concentration at the maximum, c_m, and the slope factor b. By eliminating, or making proper correction for, the effects of other variables, it should be possible to use these , alone or in combination, to detect and estimate branching in dextran polyelectrolytes.

A search of the literature was made for comparable studies involving branching and polyelectrolytes, but only two were found. Farber (102) in a characterization study of the two components of the natural polyelectrolyte carrageenan (a polygalactose sulfate) reported that the reduced viscosity-concentration curves crossed over as did the curves for our linear and branched dextran polyelectrolytes. At higher polyelectrolyte concentrations the λ fraction gave higher reduced viscosities than did the κ fraction, while at lower concentrations the reverse was true. Although Farber suggested that the two fractions might differ in respect of branching, he made no effort to prove that branching was responsible for the cross-over.

In the other investigation, Winter and Beckman (65) studied the viscosity behaviour, in pure water, of a variety of polysaccharide polyelectrolytes: carboxymethylated amylose and amylopectin (the linear and branched forms of starch) and carboxymethylated linear and branched dextrans. Making use of an extensibility factor R_m (defined as the ratio of the reduced viscosity of the polyelectrolyte at an arbitrary low concentration to the intrinsic viscosity of the polyelectrolyte in 0.0869 M HCl) they found that "for samples of equal molecular

weight the constant R_m is larger for the linear amyloses than for the branched amylopectins". Unfortunately, the carboxymethyl dextrans were not compared at the same molecular weight and the differences in R_m observed could not be -- and indeed were not -- attributed to differences in branching. (Indeed, they were attributed to the seven-fold difference in molecular weight.) It was concluded that the intensive bushy branching of amylopectin caused a difference in viscosity behaviour but that the comb-like branching in the branched dextran did not. It is possible that Winter and Beckman might have found a branching effect with their dextrans -- as we did with ours -- if they had more carefully controlled other factors (such as molecular weight) and if they had made their measurements in a dilute salt solution as solvent rather than water. In the latter event, if a cross-over were observed their criterion -- R_m -- would have quite different values depending on the arbitrary concentration at which the reduced viscosity part of the ratio was determined.

III. Summary of Contributions to Knowledge

A method for the homogeneous sulfation of dextrans -- treatment in formamide with chlorosulfonic acid at 0^oC -- has been developed which eliminates degradation

of the dextran whether by chain scission or by branch hydrolysis.

Proton magnetic resonance spectroscopy has been successfully applied to the detection and estimation of branching (non-1,6 linkages) in dextran. The method should be applicable to other polysaccharides containing more than one kind of linkage.

Proton magnetic resonance spectroscopy has also been shown to be useful in determining whether or not degradation has occurred during the sulfation of linear and branched dextran.

A characteristic cross-over of reduced viscosity concentration curves of linear and branched dextran polyelectrolytes has been demonstrated with both dextran sulfates and carboxymethyl dextrans. This effect has been shown not to be due to differences in molecular weight or molecular weight distribution or in degree of substitution but to be due primarily to differences in branching.

Two methods have been devised to demonstrate that the reduced viscosity-concentration curves all have essentially the same shape: plots of reduced viscosity against a "reduced concentration" c/c_m followed by a normalization (multiplication of η_{sp}/c values by a ratio of $\left(\eta_{sp}/c\right)_{c_m}$ values); and comparison of

"relative expansion ratios" R_n where R_n is the ratio of the $\left(\eta_{sp/c}\right)_{c_m}$ to $\eta_{sp/c}$ at concentration nc_m with n = 2, 3, 4 and 5.

It has been shown that molecular weight has no effect on the shape of the reduced viscosity-concentration curve, and branching has very little; but that degree of substitution and ionic strength of solvent both have a definite effect.

A factor which might be developed as the basis of a means of estimating branching is the slope factor b -- the slope of the straight line obtained by plotting R_n against the concentration nc_m expressed in absolute units (g./dl.). The slopes of these lines, b, have been shown to vary with ionic strength of the solvent, degree of substitution, ionogenic group and branching. Other things being equal, a branched dextran polyelectrolyte has a smaller slope factor b than does a linear dextran polyelectrolyte.

IV. Suggestions for Further Study

In this pioneering study, certain relationships have been established and indications of others found. A more exhaustive study of these relationships should prove fruitful. Since it is easier to control the degree of substitution during the preparation of the carboxymethyl dextrans, it is suggested that this polyelectrolyte

system be used in future studies.

The limit of branch detection by means of differences in the η_{sp}/c vs c curves should be investigated by using dextrans of different known degrees of branching, of the same intrinsic viscosity, and substituted to the same high degree of substitution.

Effects of molecular weight, degree of substitution and ionic strength (of solvents) should be investigated over wider ranges.

In order to establish more firmly relationships involving $\left(\eta_{sp}/c\right)_{c_m}$, c_m, and R_n, the reduced viscosity-concentration curves should be determined with great care, especially in the region of the maximum.

Once precise and accurate values of $\left(\eta_{sp}/c\right)_{c_m}$ and c_m have been obtained, values of the relative expansion ratio R_n and the slope factor b should be calculated and their relationships to the molecular weight, degree of substitution, ionic strength and branching determined over the wider ranges advocated above.

With a clearer picture of the relationships between all these and the characteristics of the reduced viscosity-concentration curves, it should then be possible to establish quantitative means of estimating branching in polyelectrolytes (or the parent polymers), independent of

molecular weight, degree of substitution, and ionic strength of the solvent.

The application of proton magnetic resonance spectroscopy in determining different kinds of glucosidic linkages should be extended to other polysaccharides (with preliminary studies on model oligosaccharides). Polysaccharides, such as starch, which are not soluble in deuterium oxide, could first be converted to polyelectrolytes.

Further study should also be made of the applicability of PRM spectroscopy to the detection and estimation of branching in other polymers (for example, addition polymers).

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G. GLOSSARY

G1 Molecular expansion factor: The average size of a flexible polymer molecule is dependent, to a large degree, upon polymer segment-solvent interactions. If segment-solvent interactions are favored, then segments of the chain repel one another and the polymer molecule occupies a greater volume. The opposite is true if polymer-solvent interactions are not favored. It is possible for the molecule to keep contracting until segments are just about touching one another, whereupon the polymer precipitates from solution. Flory and Fox (16) have introduced a function α to take into account the expansion and contraction of a polymer in different solvents. α is the ratio of the size of the molecule influenced by solvent interactions to the size the polymer molecule would have if no solvent influence existed.

G2 Unperturbed end-to-end distance: The distance between the ends of a flexible macromolecule in solution is a measure of the size of the macromolecule in the system, since this distance depends primarily upon solvent interaction and flexibility (bond angle, degree of rotational freedom, etc.). The unperturbed distance refers to the distance the ends would be separated by when there exists no solvent interaction. To illustrate:

r is the end-to-end distance of a polymer molecule; for a polymer sample, r will, of course, be an average value.

G3 Radius of gyration: If a polymer molecule is considered as an assembly of mass elements M_i, each located at a distance r_i from the center of mass, the radius of gyration (R) for a given configuration is defined by

$$R = \frac{\sum_i M_i r_i^2}{\sum_i M_i}$$

which can be transformed into a more useful function, the average radius of gyration R_G, which really is the root-mean-square radius of gyration;

$$R_G = \left(\overline{R^2}\right)^{1/2} = \left(\frac{\sum_i \overline{M_i r_i^2}}{\sum_i M_i}\right)^{1/2}$$

R_G is an average over all polymer configurations.

G4 Theta solvent (θ): A solvent for a polymer in which the polymer molecule has the same dimensions it would have in the absence of polymer-solvent interaction. A theta solvent is only a theta solvent at a particular temperature. At higher temperatures polymer-solvent interactions will occur and the polymer molecule will expand. At lower temperatures the polymer will precipitate. Under θ conditions the chain will assume random flight configurations -- the overall dimensions being determined by bond lengths and angles.

G5 Averaging: Every polymer sample has a distribution of molecular weights. Some may be very narrow while others may be very wide. Whatever the distribution, it is possible to treat the distribution statistically to obtain a mean which could serve as an overall description of the average molecular weight. It is possible to carry out the averaging process in several ways. One speaks of the "number average" if each of the molecular weights is summed and the sum divided by the number of molecules, viz.

$$\bar{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

Another way of averaging is to divide the sum of the product of the weight of the i th species and its molecular weight by the total weight,

$$\bar{M}_w = \frac{\sum_i g_i M_i}{\sum_i g_i}$$

Since the weight of the i th kind of molecule in the mixture is $g_i = \frac{N_i M_i}{\mathcal{N}}$ where N_i and M_i have the same identity as above and $\mathcal{N}$ is Avogadro's number, we can transpose to

$$\bar{M}_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M}$$

which is the weight average molecular weight defined in terms of number of particles and the molecular weight of the particle. Higher averages can be defined -- the z average

$$\bar{M}_z = \frac{\sum_i N_i M_i^3}{\sum_i N_i M_i^2}$$

and the $z + n$ average

$$\bar{M}_{z+n} = \frac{\sum_i N_i M_i^{z+n}}{\sum_i N_i M_i^{z+n-1}}$$

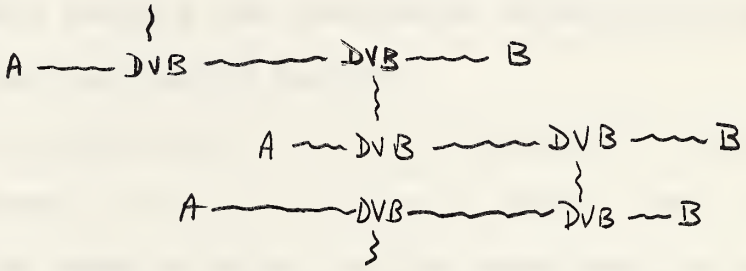
For a polymer of homogeneous molecular weight we have $M_n = M_w = M_z = M_{z+1}$ etc., while for a polymer of heterogeneous molecular weight $M_n < M_w < M_z < M_{z+1}$ etc. The various averages are used at times because of the fact that all physical properties of a polymer sample are not proportional to the same average.

G6 Effective hydrodynamic radius: The effective hydrodynamic volume of a macromolecule in solution is the volume of a solid sphere, which would present to the flowing liquid the same frictional resistance that the polymer molecule presents. The effective hydrodynamic radius then is the radius that this spherical volume would have.

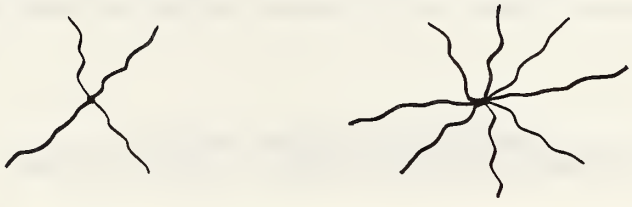
G7 Molecular friction coefficient: The sum of the contributions of the frictional coefficients of each bead or monomeric unit making up the molecule is the molecular friction coefficient.

G8 Crosslinking index: This is defined as one-half the number of branches per primary molecule, or in the Thurmond Zimm (2)

copolymerization scheme, one-half of the number of divinylbenzene (DVB) molecules per primary molecule. The primary molecule (A-B) is defined as in the diagram. For gelation, where the system is one huge molecule, the crosslinking index must then have a value of 1 or greater.



G9 Tetra and octa chain polymers: These are polymers whose branches emanate from a common point, as illustrated.



G10 Absorbance units: These are the units associated with absorbance defined in the usual way as follows:

$$A = \frac{\log (\text{intensity of beam after passing through sample})}{\log (\text{initial intensity of beam})}$$

H. APPENDIX

PMR and Preferential Sulfation

Several observations not directly pertinent to the branching study, but having to do with sulfation of dextran, have been made. The PRM spectra indicate that preferential sulfation occurs in both the linear and branched dextrans.

If each sulfate substituent were responsible for causing part of the 1,6 anomeric signal at -5.05 ppm to shift to lower field -5.40 ppm (the number of moles of sulfate substituent is less than the number of moles of anhydroglucose units (A.G.U.) (Section C III f) so that no double substitution might be expected), the ratio

$$\frac{\text{moles sulfate substituent}}{\text{moles A.G.U. - moles sulfate substituent}}$$

should be, for the linear dextran sulfate, equal to the ratio of the peak 8 area to the peak 5 area. The calculated ratio, on the basis of chemical analysis, is 2.87, whereas the experimental area ratio is 0.84 -- considerably different -- leaving no room for doubt that all of the sulfates do not contribute to peak 8. On a purely statistical basis, it would be expected that each of the secondary alcohols (carbons 2, 3, 4) would be substituted with the same probability. Thus, if not all of the sulfates cause an increased shift in the 1,6 anomeric proton signal, perhaps one-third or two-thirds do. Substituting one-third the

total number of moles of sulfate substituent into the ratio relationship, one obtains a ratio value of 0.33. When the value of two-thirds the total number of moles of sulfate substituent is substituted, the ratio value is 0.98. From these results it is obvious that a quantitative explanation of the appearance of peak 8 is not to be found on a strict statistical basis of substitution. Since the sulfate substituent is not expected to exert its influence beyond the neighboring carbon (101a) one is lead to the conclusion that only substituted secondary alcohols on carbon 2 of the ring must cause the increased chemical shift of the 1,6 anomeric proton. However, since substitution of one-third of the value of the number of moles of sulfate does not give the same ratio as the areas of the peaks, it must also be concluded that more than one-third of the total number of sulfate groups are in the 2 position. This leads to the conclusion that the hydroxyl on carbon 2 of the anhydroglucose residue has a greater reactivity than those on 3 and 4 during sulfation. As a matter of interest, a back calculation was carried out using the ratio equation above and the ratio of the areas obtained from the spectrum. This calculation indicated that 60% of the sulfate groups end up on carbon 2 in the linear dextran sulfate. The PMR data, therefore, lend further support to existing chemical evidence on the greater reactivity of carbon 2 hydroxyls in polysaccharides (103). Similar calculations on the branched

compound, assuming all the primary alcohols at the ends of the branches are completely substituted before the 2, 3 and 4 positions are substituted, indicate that 50% of the remaining sulfates end up at position 2. The reason for this lower percentage may lie in the possibility of some branches blocking position 2.

The assignments and general conclusions drawn from the linear dextran sulfate spectrum apply also to the branched dextran sulfate with one slight modification. Since the primary hydroxyls (found at the end of the branches) are more reactive than secondary hydroxyls when it comes to proton replacement (103), it can be concluded that substitution in the sulfation reaction will be predominantly at the ends of the branches where primary alcohols are found. Substitution of the primary alcohols gives a single well-defined sulfate ester function and if the protons which are attached to the carbon-bearing sulfate substituent are shifted to lower field (comparable to the shift of the protons on carbons 2, 3 or 4 when these carbons carry sulfate groups), it would be expected that a fairly well-defined peak would be found down field, not too distant from peak 1. Such a peak, 2', appears somewhat masked, on the long tail stretching between -4.0 and -4.5 ppm (Figure 23).

TABLE I

Polyelectrolyte Analytical Results

Sample	% Sulfur*	Degree of Substitution (Sulfate groups/A.G.U.)
Branched Dextran Sulfates	5.4	0.34
	8.7	0.65
	9.0	0.68
	9.5	0.74
Linear Dextran Sulfates	9.1	0.69
	10.8	0.91

	% Potassium*	Degree of Substitution (C.M.G.† /A.G.U.)
Branched Carboxymethyl Dextran	1.50	0.06
	2.90	0.13
	3.84	0.18
	4.50	0.21
	--	1.14 †≠
Linear Carboxymethyl Dextran	1.62	0.07
	3.14	0.14
	3.75	0.17
	--	1.22 †≠

	Molecular Weight	
Linear Carboxymethyl Dextran	135,000	0.82 †≠
	119,400	0.80 †≠
	73,100	0.82 †≠

* by ignition

† carboxymethyl group

≠ by direct and back titrations

TABLE II

Reduced Viscosity-Concentration Data for Branched Potassium Dextran Sulfate (5.4%S) in N/1000 KCl.

t_o = 154.91 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
218.98	1.0080	0.411
200.44	0.6720	0.438
190.45	0.5040	0.454
179.42	0.3360	0.470
173.60	0.2520	0.480
168.44	0.1833	0.475
164.70	0.1344	0.469
162.48	0.1061	0.462
160.76	0.0840	0.452
159.57	0.0695	0.432
158.81	0.0593	0.422
158.30	0.0517	0.426

TABLE III

Reduced Viscosity-Concentration Data for Branched Potassium Dextran Sulfate (8.7%S) in N/1000 KCl.

t_o = 154.85 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
237.61	0.9990	0.535
219.35	0.7215	0.578
207.93	0.5647	0.607
194.16	0.3935	0.654
186.14	0.3020	0.669
178.60	0.2239	0.683
172.78	0.1665	0.697
169.04	0.1325	0.694
166.08	0.1056	0.691
164.07	0.0878	0.683
162.61	0.0751	0.666
161.54	0.0656	0.655

TABLE IV

Reduced Viscosity-Concentration Data for Branched Potassium Dextran Sulfate (9.0%S) in N/1000 KCl.

t_o = 154.94 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
248.33	0.9710	0.621
226.43	0.6854	0.673
213.47	0.5296	0.714
197.77	0.3641	0.758
188.66	0.2774	0.786
180.36	0.2044	0.802
173.91	0.1513	0.806
169.85	0.1201	0.799
166.61	0.0955	0.785
164.44	0.0793	0.769
162.95	0.0677	0.768

TABLE V

Reduced Viscosity-Concentration Data for Branched Potassium Dextran Sulfate (9.5%S) in N/1000 KCl.

t_o = 154.91 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
247.33	0.9140	0.653
223.51	0.6093	0.727
209.92	0.4570	0.777
194.06	0.3050	0.830
185.35	0.2285	0.862
177.55	0.1662	0.878
171.62	0.1219	0.886
168.02	0.0962	0.884
165.05	0.0762	0.853
163.18	0.0630	0.841
161.80	0.0538	0.818
160.88	0.0469	0.832

TABLE VI

Reduced Viscosity-Concentration Data for Branched Potassium Dextran Sulfate (9.0%S) in N/2000 KCl.

t_o = 154.90 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
249.68	0.9060	0.675
230.56	0.6589	0.741
218.46	0.5177	0.792
197.35	0.3020	0.907
183.59	0.1858	0.996
176.61	0.1342	1.043
171.10	0.0979	1.073
167.05	0.0732	1.066
164.48	0.0584	1.062
162.78	0.0486	1.049
161.51	0.0416	1.034
160.61	0.0364	1.017

TABLE VI I

Reduced Viscosity-Concentration Data for Linear Potassium Dextran Sulfate (9.0%S) in N/1000 KCl.

t_o = 154.91 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
245.13	1.0350	0.562
226.74	0.7475	0.621
215.02	0.5850	0.663
200.49	0.4077	0.721
191.81	0.3129	0.761
183.56	0.2320	0.797
176.88	0.1725	0.823
172.63	0.1373	0.830
169.04	0.1094	0.832
166.62	0.0909	0.836
164.75	0.0778	0.823
163.45	0.0680	0.809

TABLE VIII

Reduced Viscosity-Concentration Data for Linear Potassium Dextran Sulfate (10.8%S) in N/1000 KCl.

t_o = 154.93 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
246.99	0.8920	0.666
230.11	0.6573	0.716
218.88	0.5203	0.823
204.63	0.3673	0.874
195.73	0.2838	0.927
187.02	0.2117	0.978
179.83	0.1581	1.018
175.13	0.1261	1.031
168.34	0.0838	1.038
165.15	0.0628	1.051

TABLE IX

Reduced Viscosity-Concentration Data for Linear Potassium Dextran Sulfate (9.0%S) in N/2000 KCl.

t_o = 154.81 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
246.66	0.9780	0.607
229.37	0.7113	0.677
218.23	0.5589	0.730
198.60	0.3260	0.868
185.45	0.2006	0.987
178.40	0.1449	1.052
172.88	0.1057	1.104
168.76	0.0790	1.141
166.14	0.0631	1.160
164.16	0.0525	1.151
162.80	0.0450	1.147
161.70	0.0393	1.132

TABLE X

Reduced Viscosity-Concentration Data for Linear Potassium Carboxymethyl Dextran (DS 0.07)* in N/2000 KCl.

t_o = 154.82 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
193.79	0.951	0.265
184.04	0.692	0.273
178.24	0.543	0.279
169.04	0.317	0.290
163.81	0.195	0.298
161.36	0.141	0.299
159.57	0.103	0.298
158.32	0.0768	0.294
157.51	0.0614	0.283
157.07	0.0511	0.288
156.67	0.0437	0.272

* (DS) is degree of substitution

TABLE XI

Reduced Viscosity-Concentration Data for Linear Potassium Carboxymethyl Dextran (DS 0.14) in N/2000 KCl.

t_o = 154.81 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
200.69	0.844	0.351
190.05	0.614	0.371
183.77	0.482	0.388
173.08	0.281	0.420
166.72	0.173	0.445
163.56	0.125	0.452
161.20	0.091	0.454
159.59	0.068	0.454
158.59	0.054	0.452
157.84	0.045	0.436
157.36	0.039	0.423

TABLE XII

Reduced Viscosity-Concentration Data for Linear Potassium Carboxymethyl Dextran (DS 0.17) in N/2000 KCl.

t_o = 154.77 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
210.25	0.888	0.404
197.67	0.646	0.429
190.24	0.507	0.452
177.71	0.296	0.501
170.10	0.182	0.545
166.15	0.132	0.557
163.23	0.096	0.570
161.04	0.072	0.563
159.74	0.057	0.563
158.75	0.048	0.535

TABLE XIII

Reduced Viscosity-Concentration Data for Branched Potassium Carboxymethyl Dextran (DS 0.06) in N/2000 KCl.

t_o = 154.82 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
193.54	0.888	0.282
184.20	0.646	0.294
178.47	0.5074	0.301
169.40	0.2960	0.318
164.05	0.182	0.328
161.49	0.1316	0.328
159.64	0.0960	0.324
158.29	0.0718	0.312
157.61	0.0573	0.314
157.02	0.0477	0.298
156.61	0.0408	0.284

TABLE XIV

Reduced Viscosity-Concentration Data for Branched Potassium Carboxymethyl Dextran (DS 0.13) in N/2000 KCl.

t_o = 154.81 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
207.19	0.883	0.338
199.14	0.642	0.406
187.92	0.505	0.424
175.80	0.294	0.461
168.47	0.181	0.487
164.90	0.131	0.498
162.18	0.095	0.501
160.15	0.071	0.486
158.94	0.057	0.468
158.16	0.047	0.460
157.61	0.041	0.442

TABLE XV

Reduced Viscosity-Concentration Data for Branched Potassium Carboxymethyl Dextran (DS 0.18) in N/2000 KCl.

t_o = 154.79 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
221.01	0.904	0.473
206.62	0.657	0.510
197.75	0.516	0.538
182.85	0.301	0.602
173.42	0.185	0.651
168.60	0.134	0.666
165.04	0.098	0.676
162.49	0.073	0.681
160.86	0.058	0.676
159.74	0.048	0.667
158.88	0.042	0.628

TABLE XV I

Reduced Viscosity-Concentration Data for Branched Potassium Carboxymethyl Dextran (DS 0.21) in N/2000 KCl.

t_o = 154.83 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
231.27	0.925	0.534
215.19	0.673	0.579
191.75	0.352	0.677
178.65	0.206	0.747
172.52	0.145	0.788
167.85	0.104	0.809
164.66	0.077	0.825
162.48	0.061	0.810
161.09	0.051	0.792
160.17	0.043	0.802

TABLE XVII

Reduced Viscosity-Concentration Data for Linear Potassium Carboxymethyl Dextran (DS 1.22) in N/1000 KCl,

t_o = 155.79 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
325.57	0.8510	1.281
295.20	0.6405	1.397
265.98	0.4521	1.564
245.42	0.3494	1.646
223.13	0.2402	1.799
209.54	0.1830	1.885
196.51	0.1348	1.936
186.14	0.0998	1.954
179.66	0.0792	1.932
174.36	0.0630	1.889
170.79	0.0523	1.836
168.31	0.0447	1.790
166.47	0.0390	1.768

TABLE XVIII

Reduced Viscosity-Concentration Data for Branched Potassium Carboxymethyl Dextran (DS 1.14) in N/1000 KCl.

t_o = 155.83 sec. 25.0°C

t(sec.)	c(g./dl.)	t_{sp}/c(dl./g.)
310.32	0.7970	1.195
281.07	0.6131	1.261
251.73	0.4428	1.335
220.28	0.2846	1.400
204.10	0.2097	1.421
190.18	0.1504	1.407
180.04	0.1092	1.364
174.24	0.0857	1.324
169.78	0.0675	1.280
166.86	0.0557	1.224
164.80	0.0474	1.176
163.43	0.0413	1.140

TABLE XIX

PMR Peak Assignments and Chemical Shifts of Isomaltotriose and Linear and Branched Dextran.

Spectrum	Peak	Protons Responsible	Chemical Shift (ppm)*	
Isomaltotriose	1	C_5, C_6	-3.69	
B-512 dextran	1		-3.70	
B-742 dextran	1		-3.72	av. -3.70
Isomaltotriose	2	C_2, C_3, C_4	-3.88	
B-512 dextran	2		-3.96	
B-742 dextran	2		-3.90	av. -3.91
Isomaltotriose	5	C_1 (1,6 linkage)	-5.05	
B-512 dextran	5		-5.05	
B-742 dextran	5		-5.05	av. -5.05
B-742	7	C_1 (non-1,6-linkage) branch	-5.42	av. -5.42
Isomaltotriose	4	DOH	-4.80	
B-512 dextran	4		-4.80	
B-742 dextran	4		-4.78	av. -4.80

* External standard was tetramethyl silane

TABLE XX

PMR Peak Assignments and Chemical Shifts of Dextrans and Dextran Sulfates.

Spectrum	Peak	Protons Responsible	Chemical Shift (ppm)*
B-512 dextran	1	C_5, C_6	-3.70
B-512 dextran S**	1		-3.76
B-742 dextran	1		-3.72
B-742 dextran S**	1		-3.72
B-512 dextran	2	C_2, C_3, C_4	-3.96
B-512 dextran S	2		-3.95
B-742 dextran	2		-3.90
B-742 dextran S	2		-3.92
B-512 dextran	5	C_1 (1,6 linkage)	-5.05
B-512 dextran S	5		-5.08
B-742 dextran	5		-5.05
B-742 dextran S	5		-5.06
B-512 dextran	7		--
B-512 dextran S	7	C_1 (1,6 linkage)	-5.40
B-742 dextran	7	C_1 (non-1,6-linkage)	-5.40
B-742 dextran S	7	C_1 (1,6 linkage)	
		C_1 (non-1,6-linkage)	-5.40

* External standard was tetramethyl silane

** Sulfate derivative

TABLE XXI

c_m Values for Dextran Sulfates of Same Sulfate Content

	Linear	Branched
Dextran sulfate (DS 0,7) in N/1000 KCl	0,115	0,165
Dextran sulfate (DS 0,7) in N/2000 KCl	0,063	0,085

TABLE XXII

Relative Expansion Ratios R_m for a Number of Polyelectrolyte Systems.

System	Relative Concentration	Absolute Concentration	R_n
a. Linear carboxymethyl dextran (DS 0.82) Mol. wt. 73,100 N/1000 KCl	2 c_m	0.200 g./dl.	1.09
	3	0.300	1.19
	4	0.400	1.30
	5	0.500	1.40
b. Linear carboxymethyl dextran (DS 0.80) Mol. wt. 119,400 N/1000 KCl	2	0.200	1.09
	3	0.300	1.20
	4	0.400	1.32
	5	0.500	1.43
c. Linear carboxymethyl dextran (DS 0.82) Mol. wt. 135,000 N/1000 KCl	2	0.200	1.08
	3	0.300	1.20
	4	0.400	1.31
	5	0.500	1.43
d. Linear dextran sulfate (DS 0.69) N/1000 KCl	2	0.226	1.05
	3	0.339	1.13
	4	0.452	1.18
	5	0.565	1.26
e. Branched dextran sulfate (DS 0.68) N/1000 KCl	2	0.330	1.05
	3	0.495	1.12
	4	0.660	1.19
	5	0.835	1.26
f. Linear dextran sulfate (DS 0.69) N/2000 KCl	2	0.107	1.07
	3	0.255	1.15
	4	0.340	1.22
	5	0.445	1.31
g. Branched dextran sulfate (DS 0.68) N/2000 KCl	2	0.120	1.07
	3	0.180	1.12
	4	0.240	1.24
	5	0.300	1.31
h. Linear dextran sulfate (DS 0.91) N/1000 KCl	2	0.130	1.02
	3	0.195	1.06
	4	0.260	1.11
	5	0.325	1.16
i. Linear carboxymethyl dextran (DS 1.22) N/1000 KCl	2	0.200	1.06
	3	0.300	1.14
	4	0.400	1.22
	5	0.500	1.29
j. Branched carboxymethyl dextran (DS 1.14) N/1000 KCl	2	0.364	1.04
	3	0.546	1.11
	4	0.728	1.18
	5	0.910	1.24

TABLE XXIII

The Influence of Various Factors on the Value of b.

Sample	Degree of Substitution	H_2O-KCl Solvent	b
a. Carboxymethyl dextran (linear) Mol. wt. 135,000	0.82	N/1000	1.09
b. Carboxymethyl dextran (linear) Mol. wt. 119,400	0.80	N/1000	1.09
c. Carboxymethyl dextran (linear) Mol. wt. 73,100	0.82	N/1000	1.09
d. Linear dextran sulfate	0.69	N/1000	0.65
e. Linear dextran sulfate	0.91	N/1000	0.82
f. Branched dextran sulfate	0.34	N/1000	0.26
g. Branched dextran sulfate	0.68	N/1000	0.41
h. Linear dextran sulfate	0.69	N/2000	1.30
i. Branched dextran sulfate	0.68	N/2000	0.88
j. Linear carboxymethyl dextran	1.22	N/1000	0.77
k. Branched carboxymethyl dextran	1.14	N/1000	0.37

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